INTERSPECIFIC HYBRIDIZATION IN THE GENUS DESMODIUM

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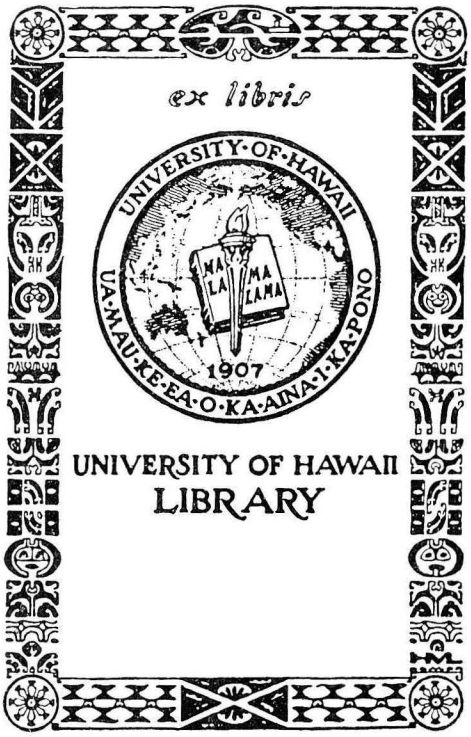
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ABSTRACT

Three species, Desmodium sandwicense E. Mey., Desmodium uncinatum (Jack.) D. C., and Desmodium intortum (Mill.) Urb., were used for interspecific hybridization studies. D. sandwicense is insensitive to daylength and flowers all year round in Hawaii, whereas D. uncinatum and D. intortum flower only in the short-day season. The percentage of pod formation through cross pollination was low, 9.2 percent for crossing two species and 4.9 percent for crossing two interspecific hybrids. Temperature greatly affected the percentage of pod formation, with increasing temperatures, the percentage of pod formation decreased. Hybrids between D. sandwicense and D. uncinatum were lower in percentage of pollen abortion than hybrids between D. sandwicense and D. intortum. Percentage of pod formation in the three species was negatively correlated with percentage of pollen abortion.

Stem color of the three species was controlled by a single pair of genes, with colored as dominant and green as recessive. The genetic behavior of internode length of Desmodium plants was controlled by multiple genes. In leaflet size, the large leaflet of D. intortum appears to be dominant to the small leaflet of D. sandwicense. In contrast

to this, the large leaflet of D. uncinatum appears to be recessive to the small leaflet of D. sandwicense. Leaflet marking on the midrib was controlled by a single pair of genes, with dominance for the marked and recessive for the non-marked. Rugose leaflet plants were found only in the three-species hybrids. It was assumed that the character was controlled by complementary genes.

One thousand-seed weights for D. sandwicense, D. intortum, and D. uncinatum were respectively 3.53, 1.84, and 4.03 grams. Results obtained from F_2 seeds indicated that seed size of Desmodium plants were governed by quantitative inheritance characteristics for its behavior.

Five esterase patterns were found among the fifteen parental clones of the three species. All the esterase zones occurring in D. uncinatum also occur in D. sandwicense. This is a good indication of a closer relationship between these two species than between D. sandwicense and D. intortum. The electrophoretic peroxidase zones were classified into four groups. The third group is identical to all the fifteen parental clones. This indicates close relationships among the three species.

The results of yield comparison showed that D. intortum had the highest green weight, and D. sandwicense, the lowest,

among the three species. In F_1 hybrids, the clone of the highest green weight was an intraspecific hybrid of D. intortum clones, I13 x I33.

INTRODUCTION

There are about 250 species in genus Desmodium. Several species, D. intortum (Mill.) Urb., D. canum (Gmel.) Schintz & Thellung, D. uncinatum (Jack.) D.C., and D. sandwicense E. Mey, have shown promise as legiminous forage crops in Hawaii. The first formal introduction of record in the Department of Agronomy and Soil Science, Hawaii Agricultural Experiment Station, for Desmodium species was D. tortuosum D.C. from New York in 1913 (Departmental introduction records). D. intortum was first introduced in 1947. The first report on culture and yield performance of two Desmodium species was made by Younge et al (68). The three species, D. sandwicense, D. uncinatum, and D. intortum, form a complex. They are crossed readily and natural hybrids have been observed in progenies from plants of the three species when grown adjacent to each other.

The purpose of this study was to investigate the breeding behavior, and the interrelationships among the three species as characterized by the seed set, percentage of pollen abortion, isozyme patterns, etc. of the F_1 and $F_1 \times F_1$ hybrids from crosses among the three species as compared with

that of their parents. In addition to this, yield comparison between the parental clones and some selected hybrid plants were made.

LITERATURE REVIEW

I. Breeding and flowering behavior of D. sandwicense, D. uncinatum and D. intortum

All three species are reported as being self-pollinated but are capable of being cross-pollinated when pollinating insects are present (34, 41, 52). Natural hybrids between D. sandwicense and D. intortum have been observed (52). Little published information is available in the literature on the breeding behavior of the three species (34, 35, 52). Hutton (34) has reported on D. uncinatum and indicated that it is self-pollinated but will outcross if environmental conditions are suitable and if pollinating insects are available. Rotar et al (52) indicated that the three species are all self-pollinated but will outcross if the opportunity is available. McWhirter (41) and Park (44) reported that relative humidity has a great effect on the success of crosses, best results were obtained under cool humid conditions. Hutton (34) reported that pollen germination of D. uncinatum was poor when the relative humidity was low.

McWhirter (41) found a factor for male sterility in D. sandwicense. D. sandwicense, when used as the male

parent in crosses with D. intortum, produced uniformly male sterile progenies. D. intortum, however, has proven to be a complete restorer, the progenies from the (D. intortum - X D. sandwicense ♂)♀ X D. intortum ♂ were completely fertile.

Male sterility is a widely distributed phenomenon in the plant kingdom. It may be caused by mechanical, morphological or environmental factors and may be implemented by genetic or cytoplasmic means (22). A number of investigations have described the morphological differences associated with male sterility in corn (36, 37, 55), sorghum (60), tobacco (5), lima beans (2), cotton (54), and winter wheat (48).

Photoperiodism, the growth response of plants to definite light and dark periods, was first described by Garner and Allard on tobacco in 1920 (26). Hanson (28) and others have demonstrated how supplemental light or darkness may be used to alter the flowering data of daylength-sensitive species to facilitate hybridization and plant breeding. Hendricks (29) has reported that the dark period has to be continuous and it exercises the chief control on flowering for short-day plants.

There is little information about the photoperiod response of Desmodium species. Under Hawaiian conditions, D. intortum and D. uncinatum are short-day plants, and

D. sandwicense is indeterminate in its flowering behavior. Wang (65) reported that a short-day photoperiod increased the flowering of D. intortum as compared with natural daylength in Taiwan. Rotar et al (52) indicated that D. sandwicense flowered under any of their combinations of long and/or short day treatments. There is no known published data on the response of D. uncinatum to photoperiod.

II. Genetics in Desmodium species

There is little published information about genetics in Desmodium species. The genus Desmodium has been shown to have 2n = 20 or 2n = 22 chromosomes. D. sandwicense, D. uncinatum and D. intortum are diploid with 2n = 22 chromosomes (51).

Flower color: Park (44) described five color classes in D. sandwicense ranging from a dark purple to a near-white. He grouped these into colored (color classes I, II, and III) and near-white (color classes IV, V-1 and V-2). He indicated that colored was dominant to near-white. The color variation was the result of quantitative differences in the amount of the anthocyanin pigment (malvidin 3, 5-diglucoside) in the flowers. Park was unable to study the inheritance of the variation within the colored or near-white group due to the interactions with unknown

flavonoid pigments and due to the difficulty of visually scoring the intermediate color classes.

When flower color is controlled by a single pair of genes, colored flowers are generally produced by a dominant gene (20). When two or more gene pairs are found, these may involve a) complementary gene action (6, 39), b) epistasis (42), or c) duplicate gene action (12). Paris and Haney (45) presented an extensive review (including 75 species) on the interaction of genes for flower color.

Stem color: Many studies on stem color have revealed a monogenic inheritance of pigmentation. Some of these are sesame (18) , cowpeas (59), kenaf (38) and okra (23). Red or yellow stems in castor bean are controlled by two independent dominant genes, R or Y over their recessive green. A pair of partial inhibitor genes, I versus i, inhibits full pigmentation producing color variation within red or yellow stems of castor bean (18). In rare cases, green stem is dominant over purple-pigmented stem, such as sweet potatoes (31) and a mutant dark-coppery red in jute (58).

Park (44) indicated that there were 3 shades of red stem color in D. sandwicense and that the red stem color was dominant to green. He was unable to determine the inheritance of the varying shades of red color. He found that

stem color was linked to flower color in coupling phase with an average recombination value of 34 percent for D. sandwicense. McWhirter (41) stated that hybrids of D. intortum, red stem, and D. sandwicense, green stem, gave segregations which indicated that stem color was controlled by a single pair of genes, with red, R, as dominant and green, r, as recessive.

Leaf marking: Brewbaker (12) and Carnahan (16) reported on a series of V-leaflet marking in white clover, which were conditioned by a multiple allelic series and were simply inherited with the recessive v for non-marking. In red clover, the presence of a central leaf spot along the midrib is determined by a dominant factor over non-marking (66). Park (44) indicated that silver marking on the midrib of leaflet of D. sandwicense was controlled by a pair of genes with the silver marking being dominant to the recessive green or non-marked.

Isozyme patterns: Starch-gel electrophoresis is one of the many biochemical techniques which may be applied as a research tool in hybridization as well as isozyme pattern determination. It is based on the principle that different biochemical molecules have different rate of migration when an electrical current is passed through the medium containing

these samples (62). Factors in the sample, which influence the rate of migration through the gel, are the charge of the sample molecules, and the size and shape of the molecules (33).

The combination of starch-gel electrophoresis with different enzyme staining methods has greatly facilitated the possibilities of studying different molecular forms of enzymes (62). Different molecular forms of an enzyme are often referred to as isozymes. The different rates of migration of these isozymes are of particular interest in regard to electrophoresis. In recent years, electrophoretic isozyme variations have been studied in a large number of different organisms. These isozymes have been found in both plants and animals, and are reported to be under genetic control (8, 25, 56, 57). The different isozyme zones and patterns also are useful in determining the affinities of species to each other.

Effects of gibberellic acid on dwarf plants: Growth of dwarf plants may be reduced about 80 percent by a defect in only one of the thousands of genes. Among these thousands of genes, there are probably several which must function properly for plants to attain normal size. A malfuction of any one of these genes may result in a dwarf

plant. No information is available in the literature about dwarfism in Desmodium species.

Phinney (46, 47) reported that application of gibberellic acid to five different single-gene dwarf mutants in corn so enhanced growth that the treated dwarf plants were very similar to the non-treated normal plants in height at the same age. Neely (43) reported that gibberellic acid could completely overcome certain dwarf characters in corn and restore plants to normal growth. Brian (13) reported that the growth rate of dwarf pea seedling shoots was significantly increased during the first four days by the application of gibberellic acid.

MATERIALS AND METHODS

I. Morphological observation on the parental clones

Five clones of D. sandwicense, numbered S11, S21, S31, S41, and S51; eight clones of D. uncinatum, numbered U12, U22, U32, U42, U52, U62, U72, and U82; and six clones of D. intortum, numbered I13, I23, I33, I43, I53, and I63 were used for interspecific hybridization in this study. A tabular summary of the morphological characteristics of the surviving fifteen clones is presented in Table 1. Several clones were lost due to virus disease.

The three species are perennials with upright to spreading growth habit. The leaves are trifoliate and the flowers are complete. The inflorescences are racemes which vary in size depending upon the species. The pods, covered with short, stiff and uncinate hairs, are very sticky and readily break into 4 to 12 segments each yielding one seed. The seed is kidney-shaped and differs considerably in size depending upon the species.

For morphological observations, the stem internode length, leaflet width and leaflet length were measured to the nearest millimeter and raceme length was measured to the nearest 0.5 cm. Leaflet hairness was determined by

counting the number of hairs under microscope within a circle of 3.2 mm diameter, and this was in turn multiplied by 12.5 as to obtain the hair number within one square centimeter. Hundred-seed weights were recorded to the nearest milligram. Stem color was confined to the internode color, and classified into three classes, green, brown and red. Growth habit was classified as upright, intermediate and spreading, and plant vigor was rated in three classes, namely, excellent, good and poor. The leaflet length X width was referred to as an index of leaflet size, and the ratio of leaflet length to width was obtained by length divided by width. Percentage of pod formation was determined by dividing the number of flowers opened on one raceme by the number of pods formed on that raceme.

II. Crosses

Desmodium species have very tiny, fragile flower buds (4-5 mm), and emasculation is difficult. In making the crosses, flower buds were emasculated in the late afternoon by opening the end of the keel petals with forceps and carefully removing the anthers from the closed buds. The racemes, bearing emasculated buds were covered with fiber pollination bags. Wet cotton was inserted at the base of

the raceme and another wet cotton ball was put inside the pollination bag to keep the humidity in the bag high. Pollination was completed the next morning between 8 and 10 A.M.

The following sets of crosses were attempted:

a) Crosses among the three species:

D. sandwicense ♀ X *D. intortum* ♂

D. sandwicense ♀ X *D. uncinatum* ♂

D. intortum ♀ X *D. uncinatum* ♂

D. uncinatum ♀ X *D. intortum* ♂

D. intortum ♀ X *D. sandwicense* ♂

(These crosses under a) above were made by Mr. Gary Wilfret during 1964-65 and turned over to me in September, 1965.)

b) The F_1's of each of the above crosses were combined as follows:

(*D. sandwicense* ♀ x *D. intortum* ♂)♀

X (*D. sandwicense* ♀ x *D. uncinatum* ♂)♂

(*D. sandwicense* ♀ x *D. uncinatum* ♂)♀

X (*D. sandwicense* ♀ x *D. intortum* ♂)♂

(*D. sandwicense* ♀ x *D. intortum* ♂)♀

X (*D. uncinatum* ♀ x *D. intortum* ♂)♂

c) The primary F_1's of a) and b) were selfed and advanced

to the F_2 generation also.

Hybrid seeds were germinated on moist filter paper in petri dishes in the laboratory and the seedlings were planted in pots in the greenhouse for two months before they were transplanted to the field at Waimanalo Farm. These were planted 6 feet apart between and within the rows. Cuttings of F_1 plants were made and grown on Campus. No backcrosses were obtained. S_1 seeds were obtained by bagging the racemes of F_1 plants and allowing the flowers to set seeds. From these S_1 seeds, F_2 progenies were obtained.

III. Breeding behavior investigations

Response to daylength, germination of hybrid seed, pollen abortion and relationship between pollen abortion, environmental conditions and pod formation were compared among the parents, F_1's, F_1 X F_1 and F_2 progenies.

Observations on flowering behavior were made on a) number of flowers opened per raceme per day; b) number of days per raceme to complete flowering; c) number of flowers opened per raceme; and d) raceme length. Percentage of pollen abortion was determined by counting at least 500 pollen grains on each of two flowers per plant. Pollen

grains were classified as normal (full and stained with acetocarmine) and shrivelled or unstained.

IV. Genetic studies

Characteristics used in genetic studies were stem color, internode length, leaflet size and silver marking on the midrib of leaflet, rugose leaflet, raceme length and seed weight. Stem color was classified into two classes, colored (red and brown) and green. Internode length was measured to the nearest 0.5 cm by sampling ten internodes from ten different stems for each plant. The fifth internode from the stem tip was measured. The middle leaflet of the fifth or sixth leaf from the stem tip was measured for length and width to the nearest millimeter. The length X width was referred to as an index of leaflet size. Silver leaflet marking was observed according to the presence or absence of the marking on the midrib of leaflet. Raceme length was measured by sampling five racemes from each plant. Measurements were made when the last few terminal flowers were opening. For seed weights, 100 seeds were weighed to the nearest milligram. These weights were multiplied by 10 to obtain 1000-seed weights.

V. Isozyme pattern determination

In running the isozyme patterns, the following solutions need to be prepared:

1) Wash solution: 5 parts methyl alcohol
5 parts water
1 part acetic acid
½ part ethyl alcohol

2) Ashton A: 2.61 g LiOH
45.18 g boric acid
3.8 liter water

3) Ashton B: 6.08 g citric acid
23.56 g Trizma mase
3.8 liter water

4) Phosphate A: 55.6 g sodium phosphate monobasic
2.0 liter water

5) Phosphate B: 107.26 g sodium phosphate monobasic
2.0 liter water

6) Tris maleate B: 25.4 g NaOH
3.8 liter water

Starch-gel preparation: Forty-eight grams of hydrolysed starch (Connaught Laboratories, Toronto, Canada) were added to 400 ml of Ashton's gel electrolyte (40 ml Ashton A and 360 ml Ashton B). The mixture is heated until the starch is dissolved, and then evaculated by suction to remove all air mixed in the solutions. The starch is then poured into the holders. These consist of plastic trays with troughs 0.3 cm deep, 4 cm wide and 26 cm long (Figure 1). One holder is capable of handling 18 samples at one time.

The power supply was operated at 300 V. The filter papers containing the extracts were removed after 20 minutes of electrophoresis. During electrophoresis, the starch gels were covered with thin polyvinyl film to prevent evaporation.

Electrophoresis was performed at room temperature, 75°F, for about 5 hours or until the front zone had migrated at least 9 cm past the point of sample insertion.

Staining methods: After the completion of the electrophoresis the starch gels were removed from the holder and incubated at 37°C for one hour in the staining solutions, and then destained and washed with a wash solution. The two different stains used to determine the different isozyme patterns are prepared as follows:

1) Esterase:
   50 ml phosphate A
   10 ml phosphate B
   40 ml water
   75 - 100 mg fast blue RR
   1 - 3 ml 1% -naphthyl acetate

   Stain for one hour at 37°C and then pour off stain, pour on wash solution, rinse, pour on more wash solution and store in wash solution for about 10 hours.

2) Peroxidase: Prepare a benzidine solution as follows:
   1 g benzidine and 9 ml water, then to equal parts of benzidine solution, add 3% hydrogen peroxide.
   Cover the gels with the solution and shake; when bright blue, add 100 ml tris maleate B.

The gels are leveled by passing a wire over the top of the holder, the excess gel is discarded.

Extraction of materials: Fresh leaves of the parental clones and F_1 hybrids were used for isozyme pattern determination. The leaves are washed in distilled water, cut into small pieces and macerated in a mortar and pestal. Small rectangular pieces of filter paper (3 X 6 mm) are saturated in the extract and inserted in a slit in the starch gel as shown in Figure 1.

Electrophoresis: Ashton A solution was put into the tank which were connected with a plastic foam wick, the inner tanks were connected to the gel holder with similar wicks. The starch-gel holder was mounted on the two tanks and covered with two plastic foam wicks as shown in Figure 1. The power supply was operated at 300 V. The filter papers containing the extracts were removed after 20 minutes of electrophoresis. During electrophoresis, the starch gels were covered with thin polyvinyl film to prevent evaportion.

Electrophoresis was performed at room temperature, 75°F, for about 5 hours or until the front zone had migrated at least 9 cm past the point of sample insertion.

Staining methods: After the completion of the electrophoresis the starch gels were removed from the holder and incubated at

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1) Esterase:
50 ml phosphate A
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40 ml water
75 - 100 mg fast blue RR
1 - 3 ml 1% -naphthyl acetate

Stain for one hour at 37°C and then pour off stain, pour on wash solution, rinse, pour on more wash solution and store in wash solution for about 10 hours.

2) Peroxidase: Prepare a benzidine solution as follows:
1 g benzidine and 9 ml water, then to equal parts of benzidine solution, add 3% hydrogen peroxide.
Cover the gels with the solution and shake; when bright blue, add 100 ml tris maleate B.

VI. Effects of gibberellic acid on dwarf D. sandwicense clones

Several dwarf Spanish clover plants were found in F_2 hybrid population within D. sandwicense crosses. Gibberellic acid was applied to the dwarf plants for overcoming dwarf characteristic and restoring normal growth. Plant No.1 and plant No.2 were sparyed with 1 ppm and 100 ppm of gibberellic acid, respectively, twice a day, plant No.3 received no gibberellic acid. After one month's treatment, measurements

on the internode length and leaflet size were made. The data were statistically analysed.

VII. Yield test

Thirty-two clones, 3 D. sandwicense clones, 3 D. uncinatum clones, 5 D. intortum clones, 5 clones of two-species hybrids, and 16 clones of three-species hybrids, were selected for yield test. The selection of hybrid plants was based on the vigor of the plants in the field. All the sixteen clones of three-species hybrids were scored for vigor. The plants for yield test were established through cuttings and planted to the field with a space of 6 feet X 6 feet per plant. The test was based on a randomized block design with 32 clones and 5 replications. Two harvests, each with an interval of 62 days, were obtained, and green weight and dry weight were recorded for statistical analysis.

RESULTS AND DISCUSSION

I. Morphological observations on the parental clones

There were nineteen clones to begin with but four of them were lost due to virus infection during the course of the studies. The clones were quite different from each other in morphology. Stem, leaf, flower, and pod and seed characteristics are summarized in Table 1.

Stem variation:

Stem growth habit ranged from an upright type to spreading type, growing along the surface of the ground and rooting at the nodes. Stem cross section varied from round, obtuse-angled to acute-angled. Stem internode length ranged from 2.9 to 8.1 cm depending upon the species and environmental conditions. Stem color varied from light green, green, brown, red to dark red depending upon the clone and/or the species. Hairness differed greatly in length, density and shape from clone to clone, some were also glandular.

a) D. sandwicense: All five clones of D. sandwicense in this study have upright growth habit, and the stems were round in cross section and the shortest of the three species. Stem internode length ranged from 2.9 to 3.2 cm. Stem color varied from green to red depending upon the

clone. D. sandwicense had few, short stem hairs which made the stem fairly smooth.

b) D. uncinatum: All the eight clones of D. uncinatum have spreading growth habit. The stems were round in cross section. The stem internode length (longest among the three species) varied from 6.2 to 8.1 cm. All the stems of D. uncinatum clones were colored, either brown or red. D. uncinatum has long, dense stem hairs with hooks at the ends, which make the stems rather sticky.

c) D. intortum: The stems of the six D. intortum clones were quite variable in morphology. They varied in growth habit, cross section type, internode length, color and hairness (Table 1). I23 had spreading growth habit, I13 and I53 had intermediate growth habit, I33 and I63 had upright growth habit (Figure 17). Internode length varied from 3.4 to 6.4 cm. I13 and I23 had red stems and the remainder of the clones were green stemmed.

Leaf variation:

Leaves of the three species are trifoliate. Leaflet size varied from species to species, and it was found that mature leaves were larger in summer, smaller in winter. The middle leaflet is always larger than the two lateral leaflets. The leaf venation of the three species is

pinnate, this is composed of one central mid-vein and many secondary veins arranged around the mid-vein. The leaflet form varied from lanceolate, elliptic to ovate depending upon the species. Leaflet apices may either be acuminate or acute and leaflet base may either be truncate or obtuse.

The leaf margin is entire, and may be provided with a row of hairs. No indentations were observed on the leaflet margins of the three Desmodium species. Silver leaflet marking on the midrib of leaflet was found in each species, but some parental clones of D. intortum did not have any silver leaflet marking on the midrib of the leaflet. Leaf color varied from light green, green to brown (Figure 2). Hairness was observed on the leaflet of each clone but differed considerably in length, density, and shape. The lower surface usually has more leaf hairs than the upper surface.

a) D. sandwicense: The leaflet-size indices (leaflet length X leaflet width) of D. sandwicense (the smallest among the three species) ranged from 8 to 12, and leaflet length to width ratios ranged from 1.74 to 1.89. The D. sandwicense clones in this study had green leaflets. Leaflet form is lanceolate, and leaflet apices are acute. The leaflet base is obtuse, and constricting abruptly to the petiole.

one-half the length. Leaflet apices are acute, and leaflet bases are truncate.

c) *D. intortum*: The leaflet-size indices of *D. intortum* ranged from 18 to 32 depending upon the clone, and leaflet length to width ratios (the widest among the three species) varied from 1.49 to 1.62. *D. intortum* clones varied in leaf color and leaflet markin, I23 had brown leaves which were not found in the other parental clones; I33, I43, I53, and I63 did not have silver marking on the midrib of leaflet. The leaflet form of *D. intortum* is ovate, with broadest below the middle. The leaflet apices may either be acute or acuminate, and leaflet bases are truncate (Figure 2).

Flowers and flowering habit:

The three species studied have complete flowers, composed of calyx, corolla, stamens and pistil. The flower color varied from nearly-white, pink to red depending upon the species and/or clones. The inflorescences of *Desmodium* plants are raceme. These varied considerably in length among the three species (Figure 13). It usually takes about two weeks for flowering to be complete on one raceme.

a) *D. sandwicense*: Flower color of *D. sandwicense* varied from nearly-white, pink to red depending upon the clone. Its racemes were about 15 cm long, and there were about 50 flowers per raceme. Flowering period per raceme averaged

b) D. uncinatum: The leaflet-size indices of D. uncinatum ranged from 21 to 27, and leaflet length to width ratios varied from 1.77 to 1.86. All the D. uncinatum clones in this study had light green leaves with silver marking on the midrib of each leaflet. Leaflet form is elliptic with the broadest point midway between the ends and the width about one-half the length. Leaflet apices are acute, and leaflet bases are truncate.

c) D. intortum: The leaflet-size indices of D. intortum ranged from 18 to 32 depending upon the clone, and leaflet length to width ratios (the widest among the three species) varied from 1.49 to 1.62. D. intortum clones varied in leaf color and leaflet marking, I23 had brown leaves which were not found in the other parental clones, I33, I43, I53, and I63 did not have silver marking on the midrib of leaflet. The leaflet form of D. intortum is ovate, with broadest below the middle. The leaflet apices may either be acute or acuminate, and leaflet bases are truncate (Figure 2).

Flowers and flowering habit:

The three species studied have complete flowers, composed of calyx, corolla, stamens and pistil. The flower color varied from nearly-white, pink to red depending upon the species and/or clones. The inflorescences of Desmodium

plants are raceme. These varied considerably in length among the three species (Figure 13). It usually takes about two weeks for flowering to be complete on one raceme.

a) D. sandwicense: Flower color of D. sandwicense varied from nearly-white, pink to red depending upon the clone. Its racemes were about 15 cm long, and there were about 50 flowers per raceme. Flowering period per raceme averaged 8,8 days, with an average of 5.8 flowers spening per raceme per day. All the clones of D. sandwicense were insensitive to daylength and bloomed the year round in Hawaii.

b) D. uncinatum: The flower color of six D. uncinatum clones studied was mallow pink. The raceme length of D. uncinatum (longer than those of D. sandwicense and D. intortum) ranged from 21.2 to 26.0 cm. The number of flowers per raceme varied from 46.8 to 48.0, and there were 3 to 4 flowers opening per raceme per day. The flowering period per raceme varied from 10.8 to 14.0 days. D. uncinatum is a short-day plant and flowered during the period October to March or April in Hawaii.

c) D. intortum: The flower color of D. intortum varied from pink to purple depending upon the clone. The raceme length ranged from 10.8 to 14.6 cm and the number of flowers on each raceme varied from 27.8 to 43.4 depending upon clones.

It took from 5.2 to 8.4 days to complete flowering per raceme, with an average of 4.3 to 5.3 flowers opened per raceme per day. D. intortum is a short-day plant flowering during the period December to March under Hawaiian conditions.

Pods and seeds:

The three species studied produce serrated pods with 4 to 12 seeds. The pods are very sticky because of harshly, hooked hairs on the surface of pods. The seed is kidney-shaped and about 1.5 mm long and 1 mm wide. Seed weight varied from species to species.

a) D. sandwicense: The percentage of pod formation of D. sandwicense was 47.2 percent. Each pod produced an average of 6.3 seeds. The 1000-seed weight of D. sandwicense was 3.54 grams. Its seed color was light brown, and the seed germination was about 99 percent for normal and mature seeds.

b) D. uncinatum: The percentage of pod formation in D. uncinatum (lower than those of D. sandwicense and D. intortum) varied from 16.9 to 32.6 percent. Each pod produced about 4 seeds. Seed weight of D. uncinatum was about 4 grams per 1000 seeds. Seed color varied from green to light brown, and the seed germination was about 96 percent for normal, mature seeds.

c) D. intortum: Percentage of pod formation in D. intortum

varied from 23.3 to 51.1 percent depending upon clones. Seeds per pod varied from 4.4 to 5.7. Seed weight was about 1.8 grams per 1000 seeds, the smallest in size among the three species. All the clones of D. intortum produced brown seed, and seed germination was about 96 percent for normal, mature seeds.

II. Breeding and flowering behavior of the three species

1. Flowering behavior and sensitivity to daylength:

Observations on flowering behavior were made on a) number of flowers per raceme; b) flowering period per raceme in days; and c) number of flowers opened per raceme per day. Results are presented in Table 2.

The three species flower in a similar manner. The day before the flowers open, the closed petals expand and are observed projecting beyond the sepals. In the late afternoon of the day before flowers open, the anthers were white or light yellow, and by about 8 P.M., the anthers start to turn yellow, indicating pollen maturation. About midnight the anthers dehisce and pollen will fall out of the anthers if disturbed. By dawn the petals have completely expanded, but the flower is still closed. Shortly after daylight, the standard petal becomes erect, and the flower is receptive

Table 2. Observations on Flowering Behavior of the Parental Clones, Their F_1 Hybrids, and F_1 X F_1 during February and March, 1967[1]

Parental clones, F_1 or F_1 X F_1 hybrids		No. of total flowers opened per raceme	Blooming period per raceme in days	No. of flowers opened per raceme per day
Parental clones:				
D. sandwicense	S31	50.8±6.5	8.8±0.9	5.77±0.61
D. uncinatum	U32	46.8±5.7	10.8±0.2	4.33±0.50
	U62	46.0±5.5	14.0±1.4	3.30±0.32
D. intortum	I23	27.8±3.1	5.2±0.5	5.30±0.56
	I43	43.3±5.3	8.4±0.4	5.17±0.87
	I53	30.8±3.2	7.2±0.4	4.27±0.51
F_1 hybrids:				
S11xI23		36.8±3.3	6.6±0.5	5.57±0.64
S31xI23		58.4±8.5	9.0±0.7	6.48±0.57
S11xU22		40.8±5.1	10.8±0.3	3.83±0.33
S11xU62		43.8±7.0	8.0±1.0	5.47±0.94
S11xU82		46.4±5.0	7.2±0.7	6.44±0.48
S31xU22		54.6±8.1	12.2±1.3	4.47±0.42
S51xU42		29.8±1.6	6.0±0.1	4.96±0.38
F_1 X F_1 Plot No.				
2213		29.0±3.3	7.8±1.1	3.72±0.32
1914		71.0±9.7	25.8±1.2	2.75±0.31
1216		53.0±4.1	15.0±1.8	3.53±0.43
2313		51.8±5.2	12.0±1.7	4.39±0.50
2215		29.0±4.9	7.4±1.4	3.91±0.70
2216		52.0±4.8	15.4±1.2	3.77±0.34
1318		43.6±5.5	14.6±1.7	2.99±0.55
2612		40.0±6.8	15.0±1.8	2.66±0.35
1817		49.0±5.1	12.0±0.9	4.08±0.48
2219		62.2±5.6	27.2±2.4	2.28±0.24
1419		30.0±2.9	10.0±1.7	2.89±0.61

Table 2. Observations on Flowering Behavior of the Parental Clones, Their F_1 Hybrids, and F_1 X F_1 during February, and March, 1967

Parental clones, F_1 or F_1 X F_1 hybrids		No. of total flowers opened per raceme		Blooming period per raceme in days		No. of flowers opened per raceme per day	
		Mean±S.E.	Range	Mean±S.E.	Range	Mean±S.E.	Range
Parental clones:							
D. sandwicense	S31	50.8±6.5	42-76	8.8±0.9	6-12	5.77±0.61	3.9-7.7
D. uncinatum	U32	46.8±5.7	28-81	10.8±0.2	8-14	4.33±0.50	3.0-5.8
	U62	46.0±5.5	34-63	14.0±1.4	9-18	3.30±0.32	2.6-4.2
D. intortum	I23	27.8±3.1	19-41	5.2±0.5	4-7	5.30±0.56	4.0-6.8
	I43	43.3±5.3	28-61	8.4 ±0.4	6-11	5.17±0.87	4.4-6.1
	I53	30.8±3.2	24-41	7.2±0.4	6-8	4.27±0.51	3.3-6.8
F_1 hybrids:							
S11xI23		36.8±3.3	28-48	6.6±0.5	5-8	5.57±0.64	4.0-7.4
S31xI23		58.4±8.5	36-77	9.0±0.7	7-11	6.48±0.57	4.5-7.7
S11xU22		40.8±5.1	28-57	10.8±0.3	8-13	3.83±0.33	2.5-5.2
S11xU62		43.8±7.0	26-62	8.0±1.0	6-11	5.47±0.94	3.1-8.7
S11xU82		46.4±5.0	28-56	7.2±0.7	5-9	6.44±0.48	5.3-8.0
S31xU22		54.6±8,1	33-75	12.2±1.3	7-20	4.47±0.42	3.7-6.3
S51xU42		29.8±1.6	27-33	6.0±0.1	5-7	4.96±0.38	3.5-5.6
F_1 X F_1 hybrids:							
(S11xI23)(S31xU22)	2213	29.0±3.3	19-36	7.8±1.1	4-10	3.72±0.32	3.2-4.7
(S21xI23)(S11xU22)	1914	71.0±9.7	56-99	25.8±1.2	23-29	2.75±0.31	2.0-4.3
(S21xI53)(S31xU22)	1216	53.0±4.1	44-67	15.0±1.8	11-20	3.53±0.43	2.2-5.0
(S21xI82)(S21xI53)	2313	51.8±5.2	31-63	12.0±1.7	8-19	4.39±0.50	3.3-6.3
(S21xI23)(S21xU72)	2215	29.0±4.9	21-48	7.4±1.4	4-11	3.91±0.70	2.1-7.0
(S21xI53)(S31xU22)	2216	52.0±4.8	41-66	15.4±1.2	12-19	3.77±0.34	2.6-4.7
(S21xI53)(U52xI33)	1318	43.6±5.5	29-59	14.6±1.7	8-15	2.99±0.55	1.9-3.9
(S31xI23)(U52xI33)	2612	40.0±6.8	26-66	15.0±1.8	9-19	2.66±0.35	1.7-3.7
(S51xI23)(S31xU22)	1817	49.0±5.1	31-60	12.0±0.9	10-15	4.08±0.48	2.1-6.0
(S51xI23)(U52xI33)	2219	62.2±5.6	44-78	27.2±2.4	21-32	2.28±0.24	1.9-3.2
(S51xI23)(S31xU22)	1419	30.0±2.9	21-38	10.0±1.7	6-7	2.89±0.61	2.0-4.7

to pollinating insects. At this time, if one trips the flower, pollen is discharged in a cloud around the stigma. Once the flowers are tripped, they quickly wilt and the standard petals are folded over the stigmas within one hour's time. Along with this a considerable change of flower color occurs from bright color to faded color.

Two flowers occur at each node and the pairs are arranged in a spiral on the raceme. Only several flowers open per raceme per day, and the two paired flowers at the same node usually open on the same day.

Twenty-seven plants, comprising 6 parental clones, 8 two-species hybrids, and 11 three-species hybrids, were observed for flowering behavior during February, and March, 1967. The number of flowers per raceme varied from 27.8 to 50.8 in the parental clones. D. sandwicense and D. uncinatum have more flowers per raceme than D. intortum. In the F_1 hybrids, the number of flowers per raceme varied from 29.0 to 71.0 depending upon the parentage and progenies examined. The average length of flowering period per raceme ranged from 5.2 to 14.0 days for the parental clones. D. uncinatum had a longer flowering period per raceme than the other two species. In F_1 hybrids, flowering period per raceme ranged from 6.0 to 27.2 days depending upon particular

plant. The number of flowers opened per raceme per day ranged from 2.3 to 6.5. There were usually 5 to 10 flowers opening per day per raceme. One reason for the low average number of flowers opened per raceme per day is that flowers did not open every day during the blooming period per raceme.

There were striking differences among the three species on flowering sensitivity to daylength. Investigations on flowering sensitivity to daylength of the parental clones and some F_1 hybrids were made monthly from September, 1966 to July, 1967 (Table 3). D. sandwicense was insensitive to daylength and flowered all year. D. uncinatum and D. intortum flowered only during the short-day season and are short-day plants. D. uncinatum may be more sensitive to daylength than D. intortum since D. uncinatum flowered during the period October to April whereas D. intortum flowered from December to March.

Clones of D. uncinatum and D. intortum growing in the field started to flower two or three weeks earlier than the same clones in the greenhouse on Campus. The plants in the greenhouse on Campus were affected by the streetlights and automobile headlights at night which delayed the onset of flowering.

An experiment to induce D. uncinatum and D. intortum

Table 3. Flowering Period of the Parental Clones Observed in the Field at Waimanalo During the Period September, 1966 to July, 1967

		Sept. 1966	Oct. 1966	Nov. 1966	Dec. 1966	Jan. 1967	Feb. 1967	March 1967	April 1967	May 1967	June 1967	July 1967
D. sandwicense	S11	***	***	***	***	***	***	***	***	***	***	***
	S21	***	***	***	***	***	***	***	***	***	***	***
	S31	***	***	***	***	***	***	***	***	***	***	***
D. uncinatum	U12		***	***	***	***	***	***	***			
	U22		***	***	***	***	***	***	***			
	U32			***	***	***	***	***	***			
	U42			***	***	***	***	***	***			
	U62		***	*****	***	***	***	***	***			
	U82		***	***	***	***	***	***	***			
D. intortum	I13				***	***	***	***				
	I23				***	***	***	***				
	I33				***	***	***	***				
	I43				***	***	***	***				
	I53				***	***	***	***				
	I63				***	***	***	***				

was made during May and June, 1966. Five D. intortum clones, I23, I33, I43, I53, I63, and two D. uncinatum clones, U12, U62, were moved into a growth chamber on May 11, 1966. The growth chamber was set at daytime temperature of 75°F, nighttime temperature of 65°F; the relative humidity varied from 85 to 90 percent; and with 10 hours of light per day. On May 29, 1966, 2 out of 4 D. uncinatum plants initiated flower buds, and flowers opened on June 3, 1966. The other two D. uncinatum plants flowered one week later. On June 21, 1966, the growth chamber became inoperative and work was discontinued. The five D. intortum clones were not induced to flower by forty days of 10-hour light. It was concluded that both species are short-day plants, D. uncinatum is more sensitive to short-day photoperiod than D. intortum.

An investigation on flowering sensitivity to daylength of the 64 three-species hybrids was made by observing the flowering behavior of the plants in the field every month during the period September, 1966 to July, 1967. The 64 hybrid plants were from the cross

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. sandwicense ♀ x D. uncinatum ♂)♂

Results are presented in Table 4 and Figure 3.

Table 4. Number of Flowering Plants, Observed Monthly During the Period September, 1966 to July, 1967, among the 64 Hybrids Obtained from the Combination of (*D. sandwicense* ♀ x *D. intortum* ♂)♀ X (*D. sandwicense* ♀ x *D. uncinatum* ♂)♂

	No. of flowering plants out of the 64 hybrid plants*
September 1966	25
October	36
November	38
December	56
January 1967	55
February	55
March	53
April	43
May	38
June	24
July	25

* Among the 64 hybrid plants, 8 plants never flowered during the observation period. These 8 plants all had short internodes and few stems and leaves.

Table 5. Pod Formation, Seed Set, and Seed Germination from Interspecific Hybridization Among Three *Desmodium* Species, *D. sandwicense*, *D. uncinatum* and *D. intortum*

Combination	No. of flowers pollinated	No. of pods formed	% of pod formation	No. of seeds set	No. of seeds germinated
D. sandwicense x *D. intortum*	507	72	14.2	271	190
D. sandwicense x *D. uncinatum*	372	57	15.3	228	82
D. intortum x *D. uncinatum*	306	7	2.3	19	7
D. uncinatum x *D. intortum*	102	5	4.9	16	15
D. intortum x *D. sandwicense*	336	8	2.4	28	12
(*D. sandwicense* x *D. intortum*) (*D. sandwicense* x *D. uncinatum*)	1593	87	5.6	247	127
(*D. sandwicense* x *D. uncinatum*) (*D. sandwicense* x *D. intortum*)	220	10	4.6	23	8
(*D. sandwicense* x *D. intortum*) (*D. uncinatum* x *D. intortum*)	933	37	4.0	110	39

2. Crosses among the three species and environmental influence on crossing behavior:

Crosses were made during the period December, 1965 to April, 1966. Flowers were emasculated in the late afternoon and were covered with fiber pollination bags containing wet cotton balls. The nest morning pollen transfers were made. Twenty to fifty flowers from 6 to 10 racemes were emasculated each afternoon from 3 P.M. to 5 P.M. Pollinations were made the next morning between 8 A.M. to 10 A.M. Sometimes, crosses were limited due to lack of desired flowers. Results of crosses among the three species are presented in Table 5.

In making two-species hybrids, 149 pods were obtained from 1623 pollinations. Percentage of pod formation was 9.18 percent. The number of seeds per pod from crosses was 3.7 which is much lower than the seeds per pod from open pollinated plants. For the three-species combination, crosses were made as follows:

A. (*D. sandwicense* ♀ x *D. intortum* ♂)♀
X (*D. sandwicense* ♀ x *D. uncinatum* ♂)♂

B. (*D. sandwicense* ♀ x *D. uncinatum* ♂)♀
X (*D. sandwicense* ♀ x *D. intortum* ♂)♂

Table 5. Pod Formation, Seed Set, and Seed Germination from Interspecific Hybridization among Three Desmodium Species, D. sandwicense, D. uncinatum, D. intortum

	No. of flowers pollinated	No. of pods formed	% of pod formation	No. of seeds/pod	No. of seeds germinated	% of seed germination
Two species:						
D. sandwicense x D. intortum	507	72	14.2	3.8	190	70.1
D. sandwicense x D. uncinatum	372	57	15.3	4.0	82	36.0
D. intortum x D. uncinatum	306	7	2.3	2.7	7	36.8
D. uncinatum x D. intortum	102	5	4.9	3.2	15	93.8
D. intortum x D. sandwicense	336	8	2.4	3.5	12	42.9
Three species:						
(D. sandwicense x D. intortum)o X (D. sandwicense x D. uncinatum)o	1593	87	5.6	2.8	127	51.4
(D. sandwicense x D. uncinatum)o X (D. sandwicense x D. intortum)o	220	10	4.6	2.3	8	34.8
(D. sandwicense x D. intortum)o X (D. uncinatum x D. intortum)o	933	37	4.0	3.0	39	35.5

C. (*D. sandwicense* ♀ x *D. intortum* ♂)♀

X (*D. uncinatum* ♀ x *D. intortum* ♂)♂

From 2716 pollinations, 134 pods were obtained with an average of 2.8 seeds per pod. Percentage of pod formation was 4.93 percent.

Although it was reported that the three species were able to cross among themselves (41, 44, 52), percentage of pod formation through cross pollination is always low. The cross compatibility among the three species in this study was estimated by the percentage of pod formation through cross-pollination (Table 5). If *D. sandwicense* is used as the female parent and crossed with *D. uncinatum* or *D. intortum*, the percentage of pod formation is relatively high, 15.3 percent for *D. sandwicense* ♀ X *D. uncinatum* ♂, and 14.2 percent for *D. sandwicense* ♀ X *D. intortum* ♂, but if *D. sandwicense* is used as the male parent, the percentage of pod formation in crosses is very low, 2.4 percent. McWhirter (41) has stated that *D. sandwicense*, when used as the male parent, in crosses with *D. intortum*, produced uniformly male sterile progenies. Results here indicate that this is probably true.

The percentage of pod formation of crosses between *D. uncinatum* and *D. intortum* was low, 2.3 percent for

D. intortum ♀ X *D. uncinatum* ♂ and 4.9 percent for *D. uncinatum* ♀ X *D. intortum* ♂ (Table 5). In crosses between F_1's X F_1's to make three-species hybrids, the average percentage of pod formation was 4.93 percent. This was much lower than the average percentage of pod formation from crossing the two species, 9.18 percent. Percentage of pod formation within the three species is influenced by environmental as well as physiological factors.

Percentage of pod formation through cross-pollinations made during the winter season 1965 to 1966 in relation to the monthly weather data for that period is presented in Table 6. Park (44) stated that best results for tripping as well as open pollination were obtained during cool weather with high relative humidity. Hutton (34) found that pollen germination of *D. uncinatum* was poor when relative humidity was low. Correspondence with Dr. McWhirter (41) also indicated that the need for high relative humidity to obtain satisfactory seed set from hand pollinations. The correlation between the percentage of pod formation through cross-pollination and the monthly average temperature was negative and statistically highly significant, r=-.977**; d.f.=3. With high temperatures the percentage of pod formation through cross-pollination decreased. The correlation

Table 6. Comparison of Percentage of Pod Formation in the Three Desmodium Species and the Monthly Average Temperature and Rainfall for the Period December, 1965 to April, 1966

	No. of pollinations	No. of pods formed	% of pod formation	Monthly average* temperature °F	Monthly rainfall in inches
December 1965	688	20	2.91	71.6	2.81
January 1966	608	36	5.92	65.1	1.39
February 1966	695	43	6.18	64.9	3.71
March 1966	513	25	4.87	67.3	0.39
April 1966	212	10	4.72	66.8	0.46
Total	2716	134			

* Local Climatological Data, University of Hawaii, Honolulu, Hawaii U.S. Weather Bureau. 1965 and 1966.

Percentage of seed germination for the parental clones was usually higher than 95 percent. The germination percentage of hybrid seeds was much lower. The average percentage of seed germination of the F_1 seeds from crosses among the three *Desmodium* species was 54.4 percent. The average percentage of seed germination of the three-species hybrids (F_1 X F_1) was 45.8 percent.

Some of the F_1 seeds germinated but produced only roots, others dried before developing the first true leaves, still others germinated and became established but failed to grow. A number of plants were lost in the greenhouse due to dessication. From 380 three-species hybrid seeds, only 174 germinated and were transplanted to pots in the greenhouse. Upon transplanting to the field at Waimanalo Farm, only 111 plants survived.

A comparative study on seed germination of three clones, *D. sandwicense* S31, *D. uncinatum* U22, and *D. intortum* I63, was made. Pictures of the germination process taken at 8 hours' intervals are presented in Figure 5. The seed was germinated at room temperature on moist blotter paper in petri dishes in the laboratory. The seed coats were slightly punctured with a sharp pin before germination.

A visible increase in seed volume was apparent at the end of the second hour. The seed coat was not always ruptured by the swelling process. At the end of the eighth

between the percentage of pod formation through cross-pollination and monthly total rainfall was not significant, r=.039; d.f.=3.

From the evidence above, it is concluded that temperature greatly affects the percentage of pod formation through cross-pollination. Temperature may influence pollen germination inside the pollen bags where wet cotton balls provide high humidity. The relationship between pod formation and temperature is presented in Figure 4.

3. Seed size and seed germination:

Since the flowering period per raceme was about two weeks long and varied from 5 to 25 days depending upon the plant, mature seed was ready to harvest on the lower part of the raceme while flowers were still in bloom. The pods shatter easily and time of harvest becomes critical. Harvesting too early produces an excessive amount of immature, shrivelled seeds. Harvesting too late causes loss of seeds. In order to minimize seed losses from immaturity or from shattering, seeds were harvested twice from each raceme, the first time only the lower pods were harvested. The remaining pods were harvested two weeks later.

The serrated pods usually contained from 4 to 12 seeds.

Seed coat color varied from green, light green, light brown to brown. Seed size varied according to the species (Table 19). The seeds contain a high percentage of hard seed. The seeds were scarified in the laboratory by puncturing the seed coat with a needle.

Percentage of seed germination for the parental clones was usually higher than 95 percent. The germination percentage of hybrid seeds was much lower. The average percentage of seed germination of the F_1 seeds from crosses among the three Desmodium species was 54.4 percent. The average percentage of seed germination of the three-species hybrids (F_1 X F_1) was 45.8 percent.

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hours' intervals are presented in Figure 5. The seed was germinated at room temperature on moist blotter paper in petri dishes in the laboratory. The seed coats were slightly punctured with a sharp pin before germination.

A visible increase in seed volume was apparent at the end of the second hour. The seed coat was not always ruptured by the swelling process. At the end of the eighth hour, the seed had swollen to its maximum volume and elongation of the hypocotyl inside the seed coat was visible. This was followed by emergence of the primary root which developed from the end of the hypocotyl. The primary root grew rapidly, about 0.4 mm per hour for D. sandwicense and D. uncinatum, 0.3 mm per hour for D. intortum. As the primary root grew downward, the seed often changed its position in the petri dish. Visible root hairs appeared when the primary root was about 0.5 cm long. After two days, the primary root was about 1.5 cm long, and the young seedlings were ready to be transplanted. All F_1 hybrid plants in this study were established in this way. At the end of the third day, the primary root was about 2.5 cm. long. It was observed that the primary root of D. intortum, I63, was shorter than those of D. sandwicense, S31, and D. uncinatum, U22. Besides this, no other obvious differences

Table 7. Percentage of Germination of Open Pollinated Seeds from the Parents, and Seeds from the Two and Three Species Crosses

Plant		% of seed germination
Parental clones:		
D. sandwicense	S21	99.5
	S31	99.0
D. uncinatum	U12	97.0
	U32	95.5
	U42	96.5
D. intortum	I13	97.0
	I33	95.5
	I63	96.5
Two-species hybrids:		
(*D. sandwicense* x *D. intortum*)		70.0
(*D. sandwicense* x *D. uncinatum*)		36.0
(*D. intortum* x *D. uncinatum*)		36.8
(*D. uncinatum* x *D. intortum*)		93.8
(*D. intortum* x *D. sandwicense*)		42.9
Three-species hybrids:		
(*D. sandwicense* x *D. intortum*) ♀ X (*D. sandwicense* x *D. uncinatum*) ♂		51.4
(*D. sandwicense* x *D. uncinatum*) ♀ (*D. sandwicense* x *D. intortum*) ♂		34.8
(*D. sandwicense* x *D. intortum*) ♀ (*D. uncinatum* x *D. intortum*) ♂		35.5

were observed.

The cotyledons would occasionally separate from the seed coat after 24 hours. Most cotyledons, however, stayed inside the seed coats for 3 to 4 days and then separated from the seed coats. After 7 days, the plumule appeared and began active growth. The cotyledons remained green for about two weeks. The discoloration of the filter paper in figures 5-b through 5-f was due to an exudate from the seed coat of the germinating seeds. Results of germination of open pollinated seeds from the parents, and seeds from the two and three species crosses are presented in Table 7.

4. Pollen abortion:

Results of pollen abortion studies are presented in Table 8 and Figure 6. Parental clones had less than seven percent shrivelled pollen. D. uncinatum had a higher percentage of shrivelled pollen grains than D. sandwicense, an average of 4.57 percent and 2.53 percent for D. uncinatum and D. sandwicense, respectively. The percentage of shrivelled pollen in D. intortum varied from 0.95 to 6.10 percent. In F_1 generations, hybrids of D. sandwicense ♀ X D. uncinatum ♂ had a low percentage of shrivelled pollen, ranging from 0.83 to 4.61 percent, whereas hybrids of D. sandwicense ♀ X D. intortum ♂ had a high percentage of

Table 7. Percentage of Germination of Open Pollinated Seeds from the Parents, and Seeds from the Two and Three Species Crosses

Plant		% of seed germination
Parental clones:		
D. sandwicense	S21	99.5
	S31	99.0
D. uncinatum	U12	97.0
	U32	95.5
	U42	96.5
D. intortum	I13	97.0
	I33	95.5
	I63	96.5
Two-species hybrids:		
(*D. sandwicense* x *D. intortum*)		70.0
(*D. sandwicense* x *D. uncinatum*)		36.0
(*D. intortum* x *D. uncinatum*)		36.8
(*D. uncinatum* x *D. intortum*)		93.8
(*D. intortum* x *D. sandwicense*)		42.9
Three-species hybrids:		
(*D. sandwicense* x *D. intortum*)♀ X (*D. sandwicense* x *D. uncinatum*)♂		51.4
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. sandwicense* x *D. intortum*)♂		34.8
(*D. sandwicense* x *D. intortum*)♀ X (*D. uncinatum* x *D. intortum*)♂		35.5

Table 8. Average of Pollen Abortion among the Three *Desmodium* Species, Their F_1 and F_1 X F_1 Hybrids

Plant	Average pollen abortion (%)
Parental clones:	
D. sandwicense	2.53
D. uncinatum	4.57
D. intortum	2.69
F_1 hybrids	
D. sandwicense x *D. uncinatum*	2.51
D. sandwicense x *D. intortum*	22.18
F_1 X F_1 hybrids:	
(*D. sandwicense* x *D. intortum*)♀ X (*D. sandwicense* x *D. uncinatum*)♂	19.33
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. sandwicense* x *D. intortum*)♂	1.83
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. uncinatum* x *D. intortum*)♂	10.99

depending upon the clones. Stem color in this study was confined to the internode color since even green stemmed plants had pigmented nodes. Although stem color could be classified into three classes, green, brown, and red, stem color in this study was recorded as either colored or green.

There was considerable difference in intensity among the colored stems. The difference in color intensity was mainly due to the effect of light intensity and age of the tissue. Observations in this study were made on older stems exposed to good light intensity, which had developed better pigmentation than new or young tissue. Results of investigations on stem color of the parent plants, F_1 hybrids and F_2 progenies and χ-square tests are presented in Table 9. Stem color of the different types are illustrated in Figure 7.

The results indicated that stem color in the *Desmodium* species studied was controlled by a single pair of genes with dominance for colored stem and recessive for green stems. One parent with colored stems and the other with green stems produced F_1 hybrids with colored stems. In the F_2 progenies, the χ-square tests indicated that the hypothesis of 3 to 1 for colored stem to green stem was not rejected. F_1 hybrids from parents with green stems were green stemmed, and F_2 hybrids from crosses among red stemmed parents were red stemmed. There was no segregation in these crosses. The results were in agreement with Park (44) who indicated that there were three shades of red stem color in *D. sandwicense*

shrivelled pollen grains, from 2.90 to 36.85 percent. This indicates that the relationship between D. sandwicense and D. uncinatum is closer than that between D. sandwicense and D. intortum, or that there is some incompatibility occurring. In the three-species hybrids, the percentage of shrivelled pollen grains varied from 0.48 to 62.17 percent. The average percentage of shrivelled pollen for 15 hybrids of

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. intortum ♀ x D. uncinatum ♂)♂

was 10.99 percent; the 31 hybrids of the combination of

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. sandwicense ♀ x D. uncinatum ♂)♂

had an average percentage of shrivelled pollen of 19.33 percent. The latter combination had more shrivelled pollen grains than the former combination.

Several parental clones grown in the greenhouse on Campus were examined for percentage of shrivelled pollen grains (Figure 6). Results indicated that clones in the greenhouse had significantly higher percentage of shrivelled pollen than the same clones grown in the field. This may be that plants in the field were grown at lower temperatures and higher relative humidities than in the greenhouse.

Pollen abortion was reported in many cases to be correlated with pod formation or male-sterility. The correlation was negative and statistically highly significant, r=-.736**, d.f.=21. It was concluded that the pod formation of Desmodium plants was negatively correlated with the percentage of shrivelled pollen grain.

III. Genetic studies

1. Stem color:

Stem color of the three species varied from dark red, red, brown, to green. All clones of D. uncinatum had red stems, D. sandwicense and D. intortum had green or red stems depending upon the clones. Stem color in this study was confined to the internode color since even green stemmed plants had pigmented nodes. Although stem color could be classified into three classes, green, brown, and red, stem color in this study was recorded as either colored or green.

There was considerable difference in intensity among the colored stems. The difference in color intensity was mainly due to the effect of light intensity and age of the tissue. Observations in this study were made on older stems exposed to good light intensity, which had developed better pigmentation than new or young tissue. Results of investigations on stem color of the parent plants, F_1 hybrids

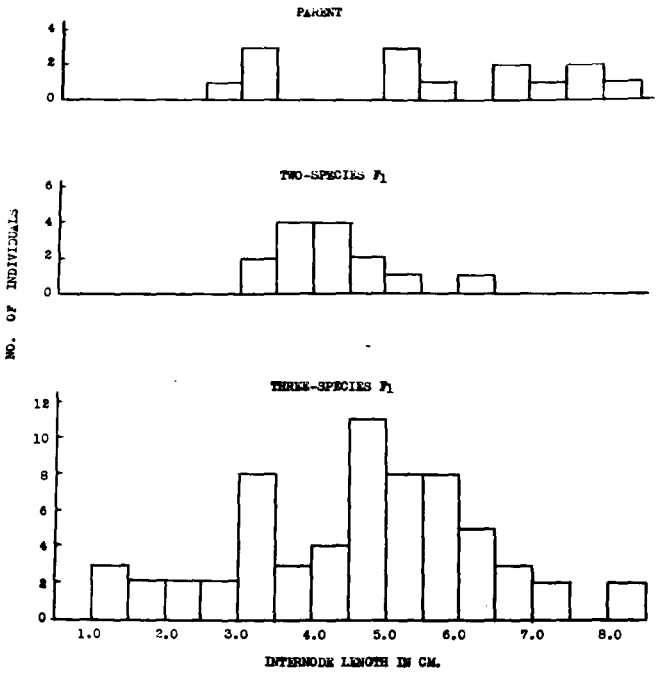
and F_2 progenies and chi-square tests are presented in Table 9. Stem color of the different types are illustrated in Figure 7.

The results indicated that stem color in the Desmodium species studied was controlled by a single pair of genes with dominance for colored stem and recessive for green stems. One parent with colored stems and the other with green stems produced F_1 hybrids with colored stems. In the F_2 progenies, the chi-square tests indicated that the hypothesis of 3 to 1 for colored stem to green stem was not rejected. F_1 hybrids from parents with green stems were green stemmed, and F_2 hybrids from crosses among red stemmed parents were red stemmed. There was no segregation in these crosses. The results were in agreement with Park (44) who indicated that there were three shades of red stem color in D. sandwicense and that the red stem color was dominant to green. He was unable to determine the inheritance of the varying shades of red color. On interspecific hybridization between Desmodium species, McWhirter (41) reported that hybrids of D. sandwicense, green stem, and D. intortum, red stem, gave segregations which indicated that stem color of these two species was controlled by a single pair of genes, with red, R, as dominant and green, r, as recessive.

Table 9. Stem Color of the Parent Plants, F_1 Hybrids and F_2 Progenies, and Chi-squares for Goodness of Fit to a 3:1 Ratio of Red to Green Stem

Cross	Stem Color of F_1's	F2 Progenies Observed		F2 Progenies Calculated		Chi-square	p
		Colored	Green	Colored	Green		
Crosses among colored							
S21xU22	colored	35	--	35	--		
S21xU82	colored	36	--	36	--		
Crosses among green							
S11xI33	green	--	34	--	34		
S11xI43	green	--	36	--	36		
S31xI43	green	--	36	--	36		
Colored X green							
S11xU22	colored	27	9	27	9	0.00	1.00
S21xU72	colored	25	11	27	9	0.60	>.25
S31xU82	colored	12	3	11.25	3.75	0.20	>.50
S11xU62*	green	--	36				
Three-species hybrids							
(S51xI23)(U52xI33) 2117	colored	25	11	27	9	0.60	>.25
(S21xI53)(U52xI33) 1218	colored	20	5	18.75	6.25	0.34	>.50
(S21xU82)(S21xI53) 2313	colored	31	5	27	9	2.36	>.10
(S21xI23)(S11xU22) 1914	colored	20	--				
(S21xI53)(S31xU22) 1216	colored	25	--				

* due to self-pollination



Distribution of Internode Length of the Parent Plants, Two-species Hybrids, and Three-species Hybrids

Figure 8. Distribution of Internode Length of the Parental Clones, F_1 and F_1 X F_1 Hybrids
Upper Diagram, Left to Right
D. sandwicense, *D. intortum*, and *D. uncinatum*

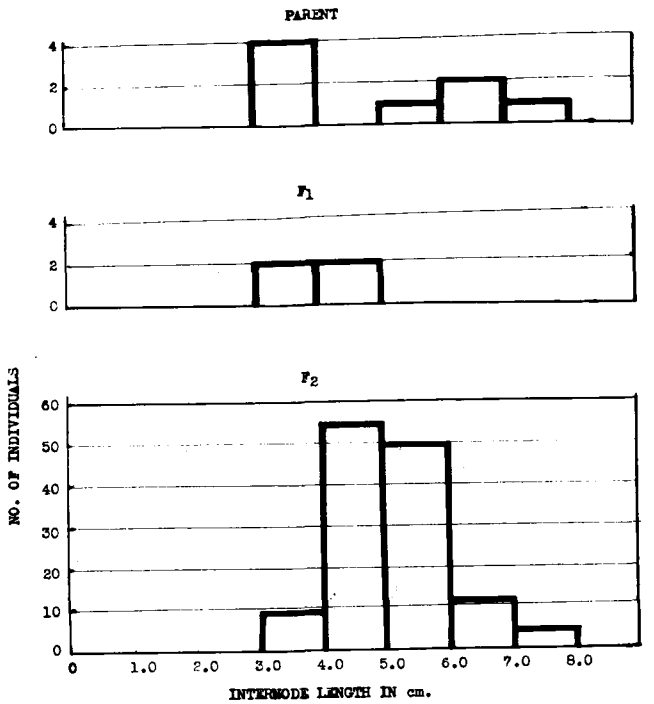
2. Internode length:

Results of internode length measurements are presented in Table 10 and Figure 8. The individual measurements are presented in Appendix Table 26. Among the parent plants, D. uncinatum had the longest internode length, ranging from 6 to 8 cm. D. sandwicense, S31, had the shortest internode length, 2.9 cm. In D. intortum, internode length varied from 3.4 to 6.3 cm depending upon the clone. Histograms showing the distribution and frequency of the internode lengths of the parent plants, two-species hybrids, and three-species hybrids are presented in Figure 8. Histograms of internode length of the two-species hybrids were intermediate to the distribution of the parents, and in the three-species hybrids, many gradations from one extreme to the other were observed.

A detailed study of internode length in the hybrids of the combination, D. sandwicense ♀ X D. uncinatum ♂, and their F_2 progenies was made in the greenhouse (Table 11). The internode length of the F_1 hybrids were always intermediate between the two parents, actually they were closer to the female parent. The distribution of the F_2 progenies were equal to that of the parents. These data are graphically presented in Figure 9.

Table 10. Average Internode Length of Stem of the Three Species, Their F_1 and F_1 X F_1 Hybrids

Plant	Average internode length in cm.
Parental clones:	
D. sandwicense	3.0
D. uncinatum	7.1
D. intortum	4.9
F_1 hybrids:	
D. sandwicense x *D. uncinatum*	3.8
D. sandwicense x *D. intortum*	4.8
D. uncinatum x *D. intortum*	5.1
D. intortum x *D. uncinatum*	4.7
F_1 X F_1 hybrids:	
(*D. sandwicense* x *D. intortum*)♀ X (*D. sandwicense* x *D. uncinatum*)♂	4.9
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. sandwicense* x *D. intortum*)♂	3.2
(*D. sandwicense* x *D. intortum*)♀ X (*D. uncinatum* x *D. intortum*)♂	4.2



Distribution of Internode Length of the Parents, F_1 Hybrids, and F_2 Progenies of D. sandwicense x D. uncinatum

Figure 9. Distribution of Internode Length of the Parents, F_1 Hybrids, and F_2 Progenies of *D. sandwicense* ♀ X *D. uncinatum* ♂
(Parent, Left to Right: *D. sandwicense* and *D. uncinatum*)

Table 11. The Internode Length of D. sandwicense, D. uncinatum, Their F_1 and F_2 Progenies

F_1's	Internode length* Parent ♀	Parent ♂	F_1	No. of F_2 progenies** 2cm	3cm	4cm	5cm	6cm	7cm	8cm	Total
S11xU22	3.2	6.6	4.1		3	19	8	6			36
S21xU72	3.0	7.3	3.8		5	27	3	1			36
S21xU82	3.0	8.1	3.6			4	26	2	4		36
S51xU42	(3.0)	7.5	4.3		1	5	13	3			22
Total					9	55	50	12	4		130

* Measurements were made in the field at Waimanalo in February, 1967.

** Measurements were made in the greenhouse on Campus in September, 1967.

3. *Leaflet size:*

The leaflet size of the three species and their F_1 hybrids was quite variable (Figure 2, page 21). Results are presented in Table 13. Individual plant measurements are presented in Appendix, Table 27.

Among the parental clones, *D. uncinatum* had an average leaflet-size index twice as large as that of *D. sandwicense*, 23.48 for *D. uncinatum* and 10.26 for *D. sandwicense*. The average leaflet-size index of *D. intortum* was 23.72, with a range from 18.44 to 32.01 depending upon the clone. A comparison of the leaflet-size indices of *D. sandwicense* ♀ X *D. intortum* ♂ and *D. sandwicense* ♀ X *D. uncinatum* ♂ crosses are presented in Table 14. In crosses of *D. sandwicense* ♀ X *D. intortum* ♂, the F_1's had leaflet-size indices as large as, or larger than that of *D. intortum*, the large leaflet parent. The large leaflet size of *D. intortum* appears to be dominant to the small leaflet size of *D. sandwicense*. In contrast to this, however, in *D. sandwicense* ♀ X *D. uncinatum* ♂ crosses, the F_1 leaflet size indices were as small as, or smaller than the small leaflet parent, *D. sandwicense*. *D. uncinatum* had the large leaflet, but in this case it appeared to be recessive to that of *D. sandwicense*. Unfortunately, none of the *D. uncinatum* ♀ X *D. intortum* ♂ hybrids survived to make these comparisons complete.

The ratio of leaflet length to width was obtained by

Results indicated that the genetic behavior of internode length of these Desmodium species was controlled by multiple genes. The multiple-gene hypothesis assumes that when two parents produce an intermediate and uniform F_1, and a variable F_2, the genes concerned are equal and cumulative in their effects. No attempt was made to determine the number of pairs of genes concerning internode length of Desmodium plant because of limited F_2 progenies in this study.

A test of independence of internode length with stem growth habit of the 64 three-species hybrid plants from the combination of

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. sandwicense ♀ x D. uncinatum ♂)♂

is presented in Table 12. Results indicated that these two characteristics were closely related. It was concluded that the plants with spreading and intermediate growth habits had significantly longer internodes than the plants with upright growth habit, (chi-square=32.79; d.f.=14; p=.01).

3. Leaflet size:

The leaflet size of the three species and their F_1 hybrids was quite variable (Figure 2, page 22). Results are presented in Table 13. Individual plant measurements are presented in Appendix, Table 27.

Table 12. Tests of Independence between Internode Length and Growth Habit of Plants from Combination of (*D. sandwicense* ♀ x *D. intortum* ♂)♀ X (*D. sandwicense* ♀ x *D. uncinatum* ♂)♂

		Internode length in cm								
		1-2cm	2-3cm	3-4cm	4-5cm	5-6cm	6-7cm	7-8cm	8-9cm	Total
Spreading	f	3	2	5	11	6	4	1	2	34
	F	2.66	2.12	5.84	8.50	8.50	4.25	1.06	1.06	
	f-F	0.34	-0.12	-0.84	2.50	-2.50	-0.25	-0.06	0.94	
Intermediate	f	0	0	0	1	10	3	1	o	15
	F	1.17	0.94	2.58	3.75	3.75	1.88	0.46	0.46	
	f-F	-1.17	-0.94	-2.58	-2.75	6.25	1.12	0.54	-0.46	
Upright	f	2	2	6	4	0	1	0	0	15
	F	1.17	0.94	2.58	3.75	3.75	1.88	0.46	0.46	
	f-F	0.83	1.06	3.42	0.25	-3.75	-0.88	-0.46	-0.46	
Total		5	4	11	16	16	8	2	2	64

Chi-square=32.79; d.f.=14; $p < .01$

Table 13. Leaflet-size Indices (Leaflet Length in cm X Width in cm) and Ratios of Leaflet Length to Width of the Three species, Their F_1 and F_1 X F_1 Hybrids

Plant	Leaflet-size Index (Length x Width)	Ratio of Length to Width
Parental clones		
D. sandwicense	10.26	1.79
D. uncinatum	23.48	1.81
D. intortum	23.72	1.56
F_1 hybrids		
D. sandwicense x *D. uncinatum*	9.77	1.75
D. sandwicense x *D. intortum*	27.43	1.60
D. intortum x *D. intortum*	31.32	1.51
F_1 X F_1 hybrids		
(*D. sandwicense* x *D. intortum*)♀ X (*D. sandwicense* x *D. uncinatum*)♂	15.03	1.74
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. sandwicense* x *D. intortum*)♂	7.53	1.75
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. uncinatum* x *D. intortum*)♂	15.22	1.72

Among the parental clones, D. uncinatum had an average leaflet-size index twice as large as that of D. sandwicense, 23.48 for D. uncinatum and 10.26 for D. sandwicense. The average leaflet-size index of D. intortum was 23.72, with a range from 18.44 to 32.01 depending upon the clone. A comparison of the leaflet-size indices of D. sandwicense ♀ X D. intortum ♂ and D. sandwicense ♀ X D. uncinatum ♂ crosses are presented in Table 14. In crosses of D. sandwicense ♀ X D. intortum ♂, the F_1's had leaflet-size indices as large as, or larger than that of D. intortum, the large leaflet parent. The large leaflet size of D. intortum appears to be dominant to the small leaflet size of D. sandwicense. In contrast to this, however, in D. sandwicense ♀ X D. uncinatum ♂ crosses, the F_1 leaflet-size indices were as small as, or smaller than the small leaflet parent, D. sandwicense. D. uncinatum had the large leaflet, but in this case it appeared to be recessive to that of D. sandwicense. Unfortunately, none of the D. uncinatum ♀ X D. intortum ♂ hybrids survived to make these comparisons complete.

The ratio of leaflet length to width was obtained by dividing leaflet length by leaflet width. D. sandwicense and D. uncinatum had leaflets narrower than those of

Table 14. Group Comparison of Leaflet-size Indices of Parents and F_1's from Crosses among D. sandwicense, D. uncinatum and D. intortum

Cross	Leaflet-size Index			Group Comparison Test		
	♀ parent	♂ parent	F_1's	t	d.f.	p
D. sandwicense X *D. intortum*						
S11xI23	10.25	18.44	28.51	4.30	18	<.01**
S21xI23	8.04	18.44	24.71	2.32	18	.025-.050*
S21xI53	8.04	26.45	28.62	0.72	18	.40-.50
S31xI23	12.58	18.14	30.72	5.01	18	<.01**
S31xI43	12.58	21.78	24.61	1.03	18	.20-.40
D. sandwicense X *D. uncinatum*						
S21xU82	8.04	21.66	7.00	0.89	18	.20-.40
S11xU62	10.15	22.64	8.53	1.36	18	.10-.20
S31xU22	12.58	21.33	11.41	0.95	18	.20-.40
S21xU72	10.15	21.56	10.38	0.21	18	.50

* significant at the 5 percent level
** significant at the 1 percent level

D. intortum, with average leaflet length to width ratios of 1.79 and 1.81 for D. sandwicense and D. uncinatum, respectively, and 1.55 for D. intortum. Pictures showing the different ratios of leaflet length to width are presented in Figure 10. In the F_1 generation, the wide leaflet of D. intortum, as well as its large leaflet size, appears to be dominant to the narrow leaflet of D. sandwicense.

In three-species hybrids, the leaflet-size index and leaflet length to width ratio varied greatly even within same combination. Some poor plants had very small leaflet-size indices and very narrow leaflets. The variation was due to segregation and recombination which occurred in the $F_1 \times F_1$.

A test of independence between leaflet-size index and plant growth habit is presented in Table 15. Results indicate that leaflet-size index was closely associated with plant growth habit. Plants with spreading and intermediate growth habit had larger leaflets than plants with upright growth habit.

4. Silver marking on the midrib of the leaflet:

There are three types of leaflet markings observable in the leaflets of these species. One is a reddish brown fleck scattered throughout the leaflets of certain D. intortum

with dominance for the markings and recessive for the non-markings. Since the three species were cross-compatible, and the evidence obtained from isozyme patterns indicated that the three species were closely related, it was assumed that the genes controlling leaflet marking on the midrib were located at one locus. This fact was also observed on stem color in the three species. It was also assumed that all the parent plants used in this study were homozygous for leaflet marking on the midrib since the three species were predominantly self-pollinated. The gene symbols, 'L' for dominant, and 'l' for recessive are proposed for the leaflet marking on the midrib of the three species. The genotype of *D. intortum* I33, I43, I53, I63 was 'll' and the rest of the parental clones were all 'LL'.

As presented in Table 16, out of 61 F_2 offsprings from the three-species hybrids, 3 were found to be non-marked. This definitely was a mistake which could be due to: 1) a few seedlings were probably mixed up during transplanting; 2) the presence of leaflet marking on the midrib was wrongly scored because for some plants, leaflet marking were merely visible on the midribs.

5. *Rugose leaflet*:

The rugose leaflet is curved backward and appears quite different from the normal leaves (Figure 11). This character is most likely due to the shortening of the midrib and

Table 15. Tests of Indipendence between Plant Growth Habit and Leaflet-size Indices of the 76 Three-species Hybrid Plants

Stem growth habit		Leaflet-size index 5-10	10-15	15-20	20-25	25-30	Total
Upright	f	11	5	2	0	0	18
	F	3	6.63	4.97	2.37	0.47	
	f-F	7.45	-1.63	-2.97	-2.37	-0.47	
Intermediate	f	0	8	4	3	1	16
	F	3.16	5.89	4.42	2.11	0.42	
	f-F	-3.16	2.11	-0.42	0.89	0.58	
Spreading	f	4	15	15	7	1	42
	F	8.29	15.47	11.61	5.53	1.11	
	f-F	-4.29	-0.47	3.39	1.47	-0.11	
Total		15	28	21	10	2	76

Chi-square=29.27; d.f.=8; $p < .01$

clones, another is the shiny appearance of the midribs and veins of the leaflet, this trait varies in its expression from non-appearance to nearly covering the leaflet. The third is the silver gray marking on the midrib of leaflet, which was observed in all three species but not in all clones.

All clones of D. sandwicense and D. uncinatum used in this study had silver marking on the midrib of leaflet. Four of the D. intortum clones were non-marked. In the F_1 hybrids of marked X marked crosses, only marked F_1 hybrids and F_2 progenies were observed. In the crosses between marked and non-marked, only marked F_1's were observed and both marked and non-marked progenies were observed in the F_2 generation. These segregated into 3 (marked) : 1 (non-marked) ratios whose chi-squares had probabilities ranging from .10 to .75 depending upon the crosses. Among the five three-species hybrids, only one segregation in a 3 to 1 ratio in F_2 generation (Table 16).

From the evidence above, it was concluded that the silver marking on the midrib of leaflet of Desmodium plant was controlled by a single pair of genes, with dominance for the marked and recessive for non-marked. This agrees with Park's conclusion (44) that silver marking on the midrib

Table 16. Leaflet-Marking on the Midribs of the Leaflets of the Parent Plants, F_1 Hybrids and S_1 Progenies

Cross	F_1 Hybrids and S_1 Progenies				X^2	p
	Observed		Calculated			
	Marked	Non-marked	3 Marked: 1 Non-marked			
Marked X Marked						
S11xU22	36	0	36	0		
S21xU22	35	0	35	0		
S21xU82	36	0	36	0		
S31xU22	36	0	36	0		
S31xU82	15	0	15	0		
Marked X Non-marked						
S11xI33	27	7	25.5	8.5	0.36	.50-.75
S11xI43	30	6	27.0	9.0	1.33	.10-.25
S31xI43	29	7	27.0	9.0	0.59	.25-.50
Three-species hybrids						
(S51xI23)(U52xI33) 2117	34	2	36	00		
(S21xI53)(U52xI33) 1218	24	1	25	0		
(S21xU82)(S21xI53) 2313	36	0	36	0		
(S21xI23)(S11xU22) 1914	20	0	36	0		
(S21xI53)(S31xU22) 1216	17	8	18.75	6.25	0.65	.10-.25

of leaflet of D. sandwicense was controlled by a pair of genes with dominance for the markings and recessive for the non-markings. Since the three species were cross-compatible, and the evidence obtained from isozyme patterns indicated that the three species were closely related, it was assumed that the genes controlling leaflet marking on the midrib were located at one locus. This fact was also observed on stem color in the three species. It was also assumed that all the parent plants used in this study were homozygous for leaflet marking on the midrib since the three species were predominantly self-pollinated. The gene symbols, 'L' for dominant, and 'l' for recessive are proposed for the leaflet marking on the midrib of the three species. The genotype of D. intortum I33, I43, I53, I63 was 'll' and the rest parental clones were all 'LL'.

As presented in Table 16, out of 61 F_2 offsprings from the three-species hybrids, 3 were found to be non-marked. This definitely was a mistake which could be due to: 1) a few seedlings were probably mixed up during transplanting; 2) the presence of leaflet marking on the midrib was wrongly scored because for some plants, leaflet markings were merely visible on the midribs.

5. Rugose leaflet:

The rugose leaflet is curved backward and appears quite different from the normal leaves (Figure 11). This character is most likely due to the shortening of the midrib and mainveins of the leaflets, causing the rugose appearance and backward curvature. Rugose leaflet character was not found among any parental clones and two-species hybrids. It was found only in the three-species hybrids of the combination (D. sandwicense ♀ x D. intortum ♂)♀ X (D. uncinatum ♀ x D. intortum ♂)♂ where D. intortum occurred twice in the combination.

Among the 19 three-species hybrid plants of the above mentioned combination, 4 plants, (S21XI53)(U52XI33) plot No. 1217, 1318; (S51XI33)(U52XI23) plot No. 2812; and (S51XI23)(U52XI33) plot No. 2020, were found to have rugose leaflets. The plants flowered from November to April like D. uncinatum. Their seed set was good and the percentage of shrivelled seed was low, 5.0 to 9.5 percent as compared with the average percentage of shrivelled seed of the three-species hybrids, 13.4 percent. The 1000-seed weights of the plants were 3.73 and 3.76 grams, about midway between those of D. uncinatum and D. sandwicense, and were much heavier than that of D. intortum, which was less than 2 grams. The internodes of these plants were short and varied from 3.1

In the F_1 X F_1 hybrids, the raceme lengths exceeded the range of the parents. The data obtained in this study were inconclusive for genetic behavior on raceme length.

There were statistically highly significant correlations between raceme length and total number of flowers per raceme, r=.542, d.f.=22; and between raceme length and length of the flowering period per raceme, r=.651, d.f.=22. This is to be expected in as much as the length of the raceme will determine the number of flowers that can be borne on it. In turn this also determines the length of the flowering period per raceme; the larger the number of flowers,the longer it will take for them to bloom.

7. *Seed weights*:

There were distinctive differences in 1000-seed weights among the three species. Results are presented in Table 19.

For the parent plants, *D. uncinatum* had the heaviest seed weights, 4.03 grams per 1000 seeds, among the three species; and the average 1000-seed weight of *D. intortum* was 1.84 grams. S31, the only clone of *D. sandwicense* available for seed weight, had 3.53 grams per 1000 seeds. These results are in agreement with Rotar and Urata's (50) who reported that *D. sandwicense* had a 1000-seed weight of 3.33 grams, seed weights of *D. uncinatum* varied from 4.00 to 4.26 grams per 1000 seeds, and the 1000-seed weights of *D. intortum* were under 2 grams.

Differences in seed size were observed in seeds from

to 5.5 cm. Their stems were densely covered with long, hooked hairs. The stem color varied from green to red. Their leaflets were medium in size, with leaflet-size indices from 12 to 16. Their leaflet hairs were dense and long, and leaflet color varied from brown to green. Their overall appearance bore partial resemblance to each of the three species, except that the rugose leaflet was never observed in any of the three species by themselves or in any F_1 hybrids among the three species. Morphological characteristics of the rugose leaflet plants are summarized in Table 17.

6. Raceme length:

Raceme length is one of the distinctive features of the three species. The flowers of Desmodium plants are borne on racemes which are indeterminate in flowering. When the basal florets are opening, the terminal florets are still in the bud stage and the racemes are relatively short. As the florets open from base to the apex, the raceme extends in length. The raceme reaches its maximum length when the last few terminal florets are opening. Measurements on raceme length was made at this stage. After this stage, the raceme shortens somewhat during the period of pod formation, and it becomes dry and considerably shortened when the seeds are mature. Five racemes were sampled from each clone for

Table 17. Characteristics of the Four Three-species Hybrids with Rugose Leaflets

	(S21xI53)(U52xI33) 1217	(S21xI53)(U52xI33)1318	(S51xI33)(U52xI23)2812	(S51xI23)(U52xI33)2020
Stem:				
Color	red	red	red	green
Internode length (cm)	5.5	4.0	3.1	3.7
Stem hairiness	dense & long	dense & long	dense & long	dense & long
Growth habit	spreading	spreading	spreading	sperading
Vigor	good	good	fair	fair
Leaflet:				
Color	green	green	brown	brown
Hairiness	dense & long	dense & long	dense & long	dense & long
Size index	15.59	12.32	13.87	15.82
Length/width	1.59	1.67	1.56	1.61
Marking	yes	yes	yes	yes
Flower:				
Color	pink	pink	light pink	pink
Pollen abortion (%)	5.5	6.9	7.8	1.3
Pod formation %	17	27	--------	22
Seed:				
1000-seed weight (g)	3.73	3.76	--------	--------

raceme length measurement. Raceme length was measured to the nearest 0.5 cm. Results are presented in Table 18.

D. uncinatum had the longest racemes among the three species, about twice as long as those of D. sandwicense or D. intortum, 24.4, 14.4 and 12.1 cm for D. uncinatum, D. sandwicense and D. intortum, respectively. In the F_1 hybrids, the raceme lengths were as short as the short raceme parents. In the F_1 X F_1 hybrids, the raceme lengths exceeded the range of the parents. The data obtained in this study were inconclusive for genetic behavior on raceme length.

There were statistically highly significant correlations between raceme length and total number of flowers per raceme, r=.542, d.f.=22; and between raceme length and length of the flowering period per raceme, r=.651, d.f.=22. This is to be expected in as much as the length of the raceme will determine the number of flowers that can be borne on it. In turn this also determines the length of the flowering period per raceme; the larger the number of flowers, the longer it will take for them to bloom.

7. Seed weights:

There were distinctive differences in 1000-seed weights among the three species. Results are presented in Table 19.

For the parent plants, D. uncinatum had the heaviest

Table 18. Average Length of the Three Species, Their F_1 and F_1 X F_1 Hybrids on Racemes

Plant	Average raceme length (cm)
Parental clones:	
D. sandwicense	14.4
D. uncinatum	24.4
D. intortum	12.1
F_1 hybrids:	
D. sandwicense x *D. uncinatum*	14.0
D. sandwicense x *D. intortum*	15.4
F_1 X F_1 hybrids:	
(*D. sandwicense* x *D. intortum*)♀ X (*D. sandwicense* x *D. uncinatum*)♂	13.9
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. sandiwcense* x *D. intortum*)♂	13.6
(*D. sandwicense* x *D. uncinatum*)♀ X (*D. uncinatum* x *D. intortum*)♂	25.7

Table 19. Thousand-seed Weights and Percentage of Shrivelled Seed from the Parent and F_1 Hybrid Plants

Plant		1000-seed weight (gr) Mean±S.E.	Shrivelled seed (%)
Parent:			
D. sandwicense	S31	3.54±.03	2.5
D. uncinatum	U12	3.99±.03	2.5
	U32	4.01±.04	3.0
	U42	4.08±.05	2.5
D. intortum	I33	1.68±.04	4.5
	I63	1.99±.03	5.0
F_1 of two species:			
(S11xU22)		2.81±.05	5.0
(S11xU62)		3.46±.02	4.5
(S21xU82)		3.00±.03	3.0
(S31xU22)		3.31±.04	6.0
(S31xU42)		3.35±.03	4.5
(S51xU42)		3.25±.06	4.0
(S31xI43)		2.53±.05	4.0
F_1 of three species:			
(S11xI23)(S31xU22)	1713	2.97±.04	15.5
	1813	2.91±.02	12.0
	1913	3.06±.04	8.0
(S11xI23)(S41xU22)	1414	2.54±.06	14.0
(S11xI23)(S11xU22)	1914	2.06±.03	17.5
(S21xI23)(S11xU22)	1915	2.52±.05	22.0
(S21xI23)(S21xU72)	2114	2.48±.04	16.0
(S21xI53)(S31xU22)	1216	1.74±.03	19.0
	1316	2.42±.04	14.0
	2216	2.31±.02	13.0
(S51xI23)(S11xU62)	1619	2.45±.03	9.5
(S21xU82)(S21xI53)	2312	3.28±.03	9.5
(S21xI53)(U52xI33)	1217	3.73±.04	9.5
(S21xI53)(U52xI33)	1318	3.76±.03	5.0

seed weights, 4.03 grams per 1000 seeds, among the three species; and the average 1000-seed weight of D. intortum was 1.84 grams. S31, the only clone of D. sandwicense available for seed weight, had 3.53 grams per 1000 seeds. These results are in agreement with Rotar and Urata's (50) who reported that D. sandwicense had a 1000-seed weight of 3.33 grams, seed weights of D. uncinatum varied from 4.00 to 4.26 grams per 1000 seeds, and the 1000-seed weights of D. intortum were under 2 grams.

Differences in seed size were observed in seeds from the hybrid plants. The reason for different seed sizes on same plant is that the seed harvested from the F_1 hybrid is actually the beginning of F_2 generation. Segregation occurs in this generation and shows many gradations in seed size. It was not possible to weigh the F_1 hybrid seeds because of limited number.

The seeds harvested from F_1 plants had a higher percentage of shrivelled seeds than those from the parent plants. This may be due to incompatibilities which prevent normal embryo and cotyledon development. Results of 't' tests indicated that the differences in percentage of shrivelled seed between the parents and the two-species hybrids were not significant ($t=1.80$, $d.f.=24$, $p>.10$).

Differences in percentage of shrivelled seed of the parents and their three-species hybrids were highly significant ($t=10.53$, d.f.=38, $p<.001$).

IV. Isozyme patterns

In an effect to trace the relationship among the parental clones of the three species, the technique of starch-gel electrophoresis in combination with two different enzyme staining methods has been applied. It was the object of this study to determine the isozyme patterns of the parental clones so as to trace the relationship among the three species. Two isozymes, esterase and peroxidase, were examined for starch-gel electrophoresis patterns in this study.

1. Esterase patterns:

The esterase zones of the three species were well separated on the starch gel. Seven electrophoretic esterase zones were observed in extracts from the leaves of the fifteen parental clones. They were called esterase zone A, B, C, D, E, F, and G in order of decreasing mobility toward the anode (Figure 12). No electrophoretic esterase zones were found to migrate toward negative pole on the starch gels. Esterase zones were classified into narrow and wide according to their size or width on the starch gel.

Figure 14. Dwarf *D. sandwicense* Plants

Esterase zone A migrates faster than all other zones and is close to the front zone. It is a wide band on the starch gel and is found only in four of D. intortum clones, I33, I43, I53, and I63. Esterase zone B is found in D. sandwicense and D. intortum clones. Esterase zone C was found in all three species but not every parental clone. It varies in width depending upon species. Esterase zone D is found only in clones of D. sandwicense. It is narrow. Esterase zone E is found in D. intortum clones, I13 and I23. Esterase zone F is found in D. sandwicense and D. intortum, and zone G is found in all the three species but not all clones. The zones differ in size, those from D. sandwicense clones are narrower and those from D. uncinatum clones are wider.

Five esterase patterns were found among the fifteen parental clones of the three species. D. sandwicense and D. uncinatum have one esterase pattern each, D. intortum has three esterase patterns.

Among the seven electrophoretic esterase zones observed on the starch gel, there is no common zone for all parental clones, that is, no one zone out of the seven appeared in all the five esterase patterns. All the esterase zones C and G occurring in D. uncinatum also occur in D. sandwicense. This

indicates that the relationship between D. sandwicense and D. uncinatum is closer than that between D. sandwicense and D. intortum. This result is in agreement with the results observed from breeding behavior among the three species in this study. It is worth noting here that all the fifteen parental clones have a similar front zone. This could be a good indication of the relationship among the three species.

2. Peroxidase patterns:

The peroxidase patterns of the three Desmodium species show quite a large variability among the fifteen parental clones. Many electrophoretic peroxidase zones were observed on the starch gels. They were classified into four groups, group A, B, C, and D, in order of decreasing mobility toward the anode (Figure 13). Only those peroxidase zones which migrated toward the anode are discussed here, since those migrating to the negative pole blurred upon staining and could not be clearly distinguished.

Group A migrates much faster than the other three groups. It consists of three zones, and occurs only in D. uncinatum clones. The middle zone is wide and distinct, whereas the two lateral zones are barely visible on the photograph of starch gel. Group B consists of four zones, and is the most important one among the four as far as isozyme

Figure 16. Young Plants of Dwarf *D. sandwicense* at Two Months of Age

pattern determination is concerned, it occurs in every parental clone but varies from one another in width and combination of the four zones. Group C consists of two zones, none has a high enoung activity to become clearly visible on the photograph of starch gel. Group D migrates very slowly, just in front of the origin. It has only one zone which is quite wide and distinct. This group was observed in all the three species except the D. intortum clones, I33, I43 and I63.

All parental clones have group-C zones which are identical. This is a good indication that the three species are related. Besides this, group B is also found in every parental clone although the zones vary greatly from one parental clone to another.

V. Effects of gibberellic acid on dwarf D. sandwicense clones

Several dwarf Spanish clover (D. sandwicense) plants were found in F_2 hybrid populations within D. sandwicense clones. They had an upright growth habit but with short internodes and short stems (Figure 14). The tallest plant among the three attained a height of 16 cm. The internodes of the dwarf plants were 0.3 cm in length. Stem color was green and the stem cross section was round. Leaflets were

Table 20. Effects of Gibberellic Acid on Internode Length and Leaflet-size Indices of Dwarf *D. sandwidense* Plants

Treatment	Internode length (cm)	Leaflet-size indices (length x width)
Control	3.4	4.6
1 ppm G.A.	4.0*	8.5**
100 ppm G.A.	6.4**	10.4**

* significantly different from the control at the 5 percent level

** significantly different from the control at the 1 percent level

very small with an average of leaflet length X width of 1.7 cm X 2.7 cm. Leaf color was dark green with very distinctive leaf marking on the midrib of leaflet (Figures 14 & 15).

The flowers of the dwarf plant was borne on very short racemes, which were about 1.2 to 2.0 cm long. The total number of flowers on one raceme varied from 16 to 25 with an average of 20 flowers. As the raceme was so short, all the flowers on one raceme were crowded together in a globose cluster (Figure 15). The plant had very poor seed set. Among the many flowers opened on the three plants, only 4 pods containing 14 seeds were harvested in 1966. The pods and seeds were normal in shape and size (Figure 15).

Germination tests for the seeds from thsee dwarf plants were done shortly after the seeds were harvested. The seed coats were slightly broken with a sharp pin before germination. Seeds were germinated at room temperature on moist blotter-paper in petri dishes. Of 14 seeds, 12 germinated, with a germination percentage of 85.7 percent. The seedlings grew very slowly for the first three months (Figure 16). Nine plants were established in the pots in the greenhouse. These were all dwarf plants just like their parents.

Gibberellic acid was applied to three dwarf D. sandwicense plants, plant No.1, plant No.2, and plant No.3.

and spreading. All the *D. sandwicense* clones and *D. intortum* clones I33 and I63 were upright in growth habit. *D. intortum* clones I13 and I53 were intermediate in growth habit. All *D. uncinatum* clones and *D. intortum* clones I23 and I43 were in spreading growth habit. Pictures showing the two different growth habits of *D. intortum* are presented in Figure 18.

Plant vigor was rated in three classes, namely, excellent, good and poor. Among the 64 three-species hybrids of the combination of

(*D. sandwicense* ♀ x *D. intortum* ♂) ♀

X (*D. sandwicense* ♀ x *D. uncinatum* ♂) ♂

29 plants were rated as excellent, 24 plants good, and 11 plants poor. Tests of independence (Table 21) indicated that growth habit was associated with vigor (χ-square=10.52, d.f.=4, p=.05). Plants with spreading or intermediate growth habit were more vigorous than the plants with upright growth habit. Of the 15 plants with intermediate growth habit, no plants of poor growth were observed.

2. *Yield comparison between the parental clones and some hybrid plants:*

Results of yield tests are presented in Table 22. Among the three species, *D. intortum* had the highest green weight, and *D. sandwicense*, the lowest. In F_1's, the hybrids of *D. sandwicense* ♀ X *D. intortum* ♂ had a higher green weight than the hybrids of *D. sandwicense* ♀ X *D. uncinatum* ♂. In

Figure 18. Growth Habits of *D. intortum* Clones
Above: Upright Growth Habit (*D. intortum* I33)
Below: Spreading Growth Habit (*D. intortum* I23)

Application were started on February 25, 1967. Plant No.1 and plant No.2 were sprayed with 1 ppm and 100 ppm of gibberellic acid, respectively, twice a day, plant No.3 received no gibberellic acid. After one month's treatment, measurements on internode length and leaflet size were made (Table 20).

No normal growth was obtained from the genetic dwarf D. sandwicense plants through the application of gibberellic acid in different concentrations. However, there was a significant increase in internode length and leaflet-size index by the application of gibberellic acid, the difference between the treatments of 1 ppm and 100 ppm gibberellic acid was also significant. It was observed that after one week of spraying with gibberellic acid, most leaves of plant No.1 and plant No.2 became yellow and started to fall down, and new leaf buds were initiated at the same time. After three weeks of spraying, there were many new leaves which were quite different in size and color from the leaves on plant No.3 which received no gibberellic acid. Those new leaves were rather light green in color (Figure 17).

VI. Yield test

1. Growth habit and vigor:

Table 20. Effects of Gibberellic Acid on Internode Length and Leaflet-size Indices of Dwarf D. sandwicense Plants

Treatment	Internode length (cm)	Leaflet-size indices (length x width)
Control	3.4	4.6
1 ppm G.A.	4.0*	8.5**
100 ppm G.A.	6.4**	10.4**

* significantly different from the control at the 5 percent level

** significantly different from the control at the 1 percent level

F_1 X F_1 hybrids, the plants of

(*D. sandwicense* ♀ x *D. intortum* ♂) ♀

X (*D. uncinatum* ♀ x *D. intortum* ♂) ♂

had a very high green weight, higher than their parents. The clone of highest green weight was an intraspecific hybrid of *D. intortum* clones, Ill X I33.

On dry matter percentage, *D. sandwicense* and *D. uncinatum* clones were higher than *D. intortum*. In F_1's, the hybrids of *D. sandwicense* ♀ X *D. uncinatum* ♂ had higher dry matter percentage than the hybrids of *D. sandwicense* ♀ X *D. intortum* ♂. It was observed that the plants with higher green weight yield had lower dry matter percentage.

The tests of comparisons among means of green weights and dry matter percentages are presented in Table 23 and Table 24, respectively. The brackets in the tables indicate that there are no differences at 5 percent significant level.

It was observed that in *D. uncinatum* clones, the green weight decreased, and the dry matter percentage increased markedly in the second harvest as compared with those of the first harvest. The reason for this phenomenon is that *D. uncinatum* started to flower at the beginning of October, this greatly reduced its vegetative growth and caused some stems and leaves to dry out.

Gowth habit was classified as upright, intermediate, and spreading. All the D. sandwicense clones and D. intortum clones I33 and I63 were upright in growth habit. D. intortum clones I13 and I53 were intermediate in growth habit. All D. uncinatum clones and D. intortum clones I23 and I43 were in spreading growth habit. Pictures showing the two different growth habits of D.intortum are presented in Figure 18.

Plant vigor was rated in three classes, namely, excellent, good and poor. Among the 64 three-species hybrids of the combination of

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. sandwicense ♀ x D. uncinatum ♂)♂

29 plants were rated as excellent, 24 plants good, and 11 plants poor. Tests of independence (Table 21) indicated that growth habit was associated with vigor (chi-square =10.52, d.f.=4, p=.05). Plants with spreading or intermediate growth habit were more vigorous than the plants with upright growth habit. Of the 15 plants with intermediate growth habit, no plants of poor growth were observed.

2. Yield comparison between the parental clones and some hybrid plants:

Results of yield tests are presented in Table 22. Among the three species, D. intortum had the highest green weight,

Table 24. Dry Matter Percentage of the 32 Clones and Tests of Comparisons among Means from Two Harvests Taken in September and November of 1967

Clone	Dry matter percentage		
	1st harvest	2nd harvest	average
U42	25.6	37.0	31.3
U12	25.2	36.8	31.0
U62	26.5	34.4	30.5
S11	29.1	31.7	30.4
S31	28.0	30.2	29.1
S21	27.8	29.1	28.5
S31xU42	27.7	29.1	28.4
I23	27.8	26.9	27.4
(S11xI23)(S31xU22) 1213	23.9	29.7	26.6
(S51xI23)(S31xU22) 1818	25.3	27.4	26.4
(S21xI53)(S31xU22) 2016	24.9	27.6	26.3
(S11xI23)(S31xU22) 1812	24.7	27.4	26.1
(S21xI53)(S31xU22) 1316	24.3	27.7	26.0
(S21xI23)(S21xU72) 2014	25.0	26.7	25.8
(S51xI23)(S31xU22) 1819	24.2	27.1	25.6
(S51xI23)(S31xU22) 1919	25.2	25.5	25.4
S31xI53	24.3	26.2	25.3
I63	25.4	24.5	25.0
S11xI23	24.5	25.3	24.9
I13	25.4	24.3	24.8
(S11xI23)(S31xU22) 1512	24.4	24.7	24.6
(S51xI23)(S31xU22) 1717	23.5	25.4	24.5
I43	25.3	23.2	24.3
(S21xI53)(S31xU22) 1216	23.3	25.3	24.3
(S21xI23)(S11xU22) 2015	23.2	25.3	24.2
(S11xI23)(S31xU22) 1813	24.1	24.3	24.2
(S51xI23)(S31xU22) 1618	22.8	24.7	23.8
S21xI23	23.0	24.1	23.6
(S21xI23)(S21xI72) 2214	23.1	24.1	23.6
(S21xI23)(U52xI33)	21.4	24.9	23.2
I13xI33	22.9	23.4	23.2
I53	22.9	23.2	23.1

D=0.8998∿0.9 at 5% significant level

Table 21. Tests of Independence between Plant Growth Habit and Vigor of the 64 Plants from Combination (*D. sandwicense* ♀ x *D. intortum* ♂)♀ X(*D. sandwicense* ♀ x *D. uncinatum* ♂)♂

		Vigor classification			
		excellent	good	poor	total
Upright growth	f	3	6	6	15
	F	6.80	5.62	2.58	15
	f-F	-3.80	0.38	3.42	
Intermediate growth	f	8	7	0	15
	F	6.80	5.62	2.58	
	f-F	1.20	1.38	-2.58	
Spreading growth	f	18	11	5	34
	F	15.41	12.75	5.84	
	f-F	2.59	-1.75	-0.84	
Total		29	24	11	64

Chi-square=10.52; d.f.=4; $p < .05$

Table 22. Average Green Weight and Dry Matter Percentage of the Three Species, Their F_1 and F_1 X F_1 Hybrids

Plant	Green Weight (grams/62 days)	Dry Matter Percentage
D. sandwicense	73.4	29.3
D. uncinatum	129.9	30.9
D. intortum	234.9	24.9
F_1 hybrids:		
D. sandwicense ♀ X *D. uncinatum* ♂	90.9	28.4
D. sandwicense ♀ X *D. intortum* ♂	359.1	24.5
D. intortum ♀ X *D. intortum* ♂	612.4	23.2
F_1 X F_1 hybrids:		
(*D. sandwicense* ♀ x *D. intortum* ♂)♀ X (*D. sandwicense* ♀ x *D. uncinatum* ♂)♂	262.1	25.2
(*D. sandwicense* ♀ x *D. intortum* ♂)♀ X (*D. uncinatum* ♀ x *D. intortum* ♂)♂	546.6	24.9

and D. sandwicense, the lowest. In F_1's, the hybrids of of D. sandwicense ♀ X D. intortum ♂ had a higher green weight than the hybrids of D. sandwicense ♀ X D. uncinatum ♂. In F_1 X F_1 hybrids, the plants of

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. uncinatum ♀ x D. intortum ♂)♂

had a very high green weight, higher than their parents. The clone of highest green weight was an intraspecific hybrid of D. intortum clones, I11 X I33.

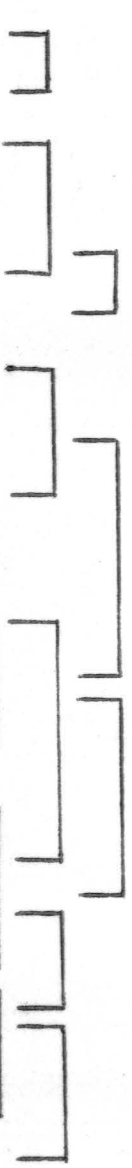
On dry matter percentage, D. sandwicense and D. uncinatum clones were higher than D. intortum. In F_1's, the hybrids of D. sandwicense ♀ X D. uncinatum ♂ had higher dry matter percentage than the hybrids of D. sandwicense ♀ X D. intortum ♂. It was observed that the plants with higher green weight yield had lower dry matter percentage.

The tests of comparisons among means of green weights and dry matter percentages are presented in Table 23 and Table 24, respectively. The brackets in the tables indicate that there are no differences at 5 percent significant level.

It was observed that in D. uncinatum clones, the green weight decreased, and the dry matter percentage increased markedly in the second harvest as compared with those of the first harvest. The reason for this phenomenon is that

Table 23. Green Weights in Gram of the 32 Clones and Tests of Comparisons among Means from Two Harvests Taken in September and November of 1967

Clone	Green weight in gram		
	1st	2nd	average
I13xI33	517.8	707.0	612.4
I53	469.4	664.8	567.1
(S21xI23)(U52xI33)	571.2	522.0	546.6
S21xI53	382.6	488.6	435.6
S11xI23	319.2	505.6	412.4
(S51xI23)(S31xU22) 1618	415.8	407.0	411.4
(S51xI23)(S31xU22) 1819	382.0	406.6	394.3
(S11xI23)(S31xU22) 1512	382.4	461.8	372.1
(S21xI23)(S21xU72) 2214	324.0	350.0	337.0
I13	142.8	493.2	318.0
(S51xI23)(S31xU22) 1717	421.2	180.8	301.0
(S11xI23)(S31xU22) 1812	306.4	285.8	296.1
(S21xI53)(S31xU22) 2016	277.6	234.4	256.0
U12	446.0	53.6	249.8
S31xI53	143.2	315.4	229.3
(S51xI23)(S31xU22) 1919	141.8	310.4	226.1
(S21xI53)(S51xU22) 1216	185.2	264.6	224.9
(S11xI23)(S31xU22) 1213	292.0	118.6	205.3
(S21xI23)(S11xU22) 2015	182.4	227.0	204.7
(S21xI53)(S31xU22) 1316	220.4	181.2	200.8
(S21xI23)(S11xU22) 2014	176.8	223.6	200.2
U64	285.8	54.8	170.3
(S11xI23)(S31xU22) 1813	187.8	142.0	164.9
U42	268.0	49.4	158.7
I43	82.8	214.4	148.6
(S51xI23)(S31xU22) 1818	82.0	191.0	136.5
S21	108.6	159.2	133.9
S31xU42	67.2	114.6	90.6
I63	41.0	102.4	71.7
I23	46.8	91.8	69.3
S11	50.6	56.0	53.3
S31	31.2	34.6	32.9



D=52.3 at 5% level of significance (Snedecor's Statistical Methods, 5th ed. P. 251).

Table 24. Dry Matter Percentage of the 32 Clones and Tests of Comparisons among Means from Two Harvests Taken in September and November of 1967

Clone	Dry matter percentage		
	1st harvest	2nd harvest	average
U42	25.6	37.0	31.3
U12	25.2	36.8	31.0
U62	26.5	34.4	30.5
S11	29.1	31.7	30.4
S31	28.0	30.2	29.1
S21	27.8	29.1	28.5
S31xU42	27.7	29.1	28.4
I23	27.8	26.9	27.4
(S11xI23)(S31xU22) 1213	23.9	29.7	26.6
(S51xI23)(S31xU22) 1818	25.3	27.4	26.4
(S21xI53)(S31xU22) 2016	24.9	27.6	26.3
(S11xI23)(S31xU22) 1812	24.7	27.4	26.1
S21xI53)(S31xU22) 1316	24.3	27,7	26.0
(S21xI23)(S21xU72) 2014	25.0	26.7	25.8
(S51xI23)(S31xU22) 1819	24.2	27.1	25.6
(S51xI23)(S31xU22) 1919	25.2	25.5	25.4
S31xI53	24.3	26.2	25.3
I63	25.4	24.5	25.0
S11xI23	24.5	25.3	24.9
I13	25.4	24.3	24.8
(S11xI23)(S31xu22) 1512	24.4	24.7	24.6
(S51xI23)(S31xU22) 1717	23.5	25.4	24.5
I43	25.3	23.2	24.3
(S21xI53)(S31xU22) 1216	23.3	25.3	24.3
(S21xI23)(S11xU22) 2015	23.2	25.3	24.2
(S11xI23)(S31xU22) 1813	24.1	24.3	24.2
(S51xI23)(S31xU22) 1618	22.8	24.7	23.8
S21xI23	23.0	24.1	23.6
(s21xI23)(S21xI72) 2214	23.1	24.1	23.6
(S21xI23)(U52xI33)	21.4	24.9	23.2
I13xI33	22.9	23.4	23.2
I53	22.9	23.2	23.1

D=0.8998≒0.9 at 5% significant level

D. uncinatum started to flower at the beginning of October, this greatly reduced its vegetative growth and caused some stems and leaves to dry out.

SUMMARY AND CONCLUSIONS

The main purpose of this thesis was to study breeding and flowering behavior, genetics, and isozyme patterns of the three species, D. sandwicense, D. uncinatum, and D. intortum. D. sandwicense was insensitive to daylength and flowered all year round in Hawaii. D. uncinatum and D. intortum flowered only in the short-day season, from October to April and December to March, respectively. D. uncinatum was induced to flower by 18 days of short-day photoperiod, whereas D. intortum did not flower even by 40 days of short-day photoperiod induction.

In crosses, the percentage of pod formation was low, 9.2 percent for crossing two-species to make F_1's, and 4.9 percent for F_1 X F_1 to make three-species hybrids. Results indicated that if D. sandwicense was used as the female parent and crossed with D. uncinatum or D. intortum, the percentage of pod formation was relatively high, 15.3 percent for D. sandwicense ♀ X D. uncinatum ♂, and 14.2 percent for D. sandwicense X D. intortum ♂, but if D. sandwicense was used as the male parent, the percentage of pod formation in crosses was very low, 2.4 percent. The percentage of pod formation in crosses between D. uncinatum and D. intortum was 4.9 percent, for

D. uncinatum ♀ X *D. intortum* ♂, and 2.3 percent for *D. intortum* ♀ X *D. uncinatum* ♂.

In addition to low percentages of pod formation from crosses, germination percentages of hybrid seeds were also very low, 54.5 percent for F_1 seeds and 45.8 percent for F_1 X F_1 seeds. Hybrids of *D. sandwicense* ♀ X *D. uncinatum* ♂ had a low percentage of pollen abortion, 2.51 percent; whereas hybrids of *D. uncinatum* ♀ X *D. intortum* ♂ had 22.18 percent of pollen abortion. It was concluded that the relationship between *D. sandwicense* and *D. uncinatum* was closer than that between *D. sandwicense* and *D. intortum*. Pod formation in the three species was negatively correlated to the pollen abortion.

Stem color of the three species was controlled by a single pair of genes, with colored, red and brown, as dominant and green as recessive. Internode lengths of F_1 hybrids were intermediate between the two parents; and many gradations from one extreme to the other were observed in F_1 X F_1 hybrids. It was concluded that the genetic behavior of internode length of the three species was controlled by multiple genes. Internode length was associated with growth habit, plants with upright growth habit had shorter internodes.

D. uncinatum and *D. intortum* clones had leaflet-size

indices (leaflet length X width) twice as large as those of D. sandwicense clones. In F_1 hybrids, the large leaflet of D. intortum appeared to be dominant to the small leaflet of D. sandwicense. In contrast to this, the large leaflet of D. uncinatum appeared to be recessive to the small leaflet of D. sandwicense.

Silver markings on the midribs of leaflets were observed in each species. Results in this study indicated that this marking on the midrib of the leaflets in the three species was controlled by a single pair of genes, with dominance for the marked and recessive for the non-marked leaflets.

Rugose leaflet plants were found in the three-species hybrids of the combination

(D. sandwicense ♀ x D. intortum ♂)♀

X (D. uncinatum ♀ x D. intortum ♂)♂.

D. uncinatum had racemes about twice as large as those of D. sandwicense and D. intortum. In the F_1 hybrids, the raceme lengths were as short as the short raceme parents. In the F_1 X F_1 hybrids, the raceme lengths exceeded the range of the parents.

One thousand-seed weights for D. sandwicense, D. uncinatum and D. intortum were 3.53, 4.03, and 1.84 grams, respectively. Results indicated that seed size of Desmodium

plants was likely governed by quantitative inheritance characteristics for its behavior.

Seven electrophoretic esterase zones were found on starch gels according to the mobility toward the anode. Five esterase patterns were found among the fifteen parental clones of the three species. All esterase zones occurring in D. uncinatum occurred in D. sandwicense. Over ten electrophoretic peroxidase zones, classified into four groups, were found on the starch gel. Group B, consisting of four zones, is the most important one among the four groups and occurs in every parental clone, but differs among clones in width and combination of the zones. Group C consists of two zones and is the same in all parental clones.

No normal growth was obtained from the genetic dwarf D. sandwicense plants through the application of gibberellic acid in different concentrations. However, there was a significant increase in internode length and leaflet-size index by the application of gibberellic acid.

Plant vigor was found to be associated with growth habit, plants with spreading or intermediate growth habit were more vigorous than plants with upright growth habit. The results of yield tests indicated that D. intortum had the highest green weight, and D. sandwicense, the lowest

among the three species. Hybrids of D. sandwicense ♀ X D. intortum ♂ had a higher green weight than the hybrids of D. sandwicense ♀ X D. uncinatum ♂. Plants of the combination (D. sandwicense ♀ x D. intortum ♂)♀

X (D. uncinatum ♀ x D. intortum ♂)♂

had higher green weights than those of their parents. Among the 32 clones selected for yield comparison, the clone with the highest green weight was an intraspecific hybrid of D. intortum clones, I13 X I33.

APPENDIX

Table 25. Percentage of Pollen Abortion among the Three *Desmodium* Species and Their Hybrids

Plant		Pollen Abortion (%) Mean±S.E.
Parent plants:		
D. sandwicense	S11	4.03±0.31
	S21	1.92±0.21
	S31	1.65±0.22
D. uncinatum	U12	4.08±0.15
	U32	6.96±1.03
	U42	3.31±0.53
	U62	3.84±0.63
D. intortum	I23	6.10±0.68
	I43	0.95±0.22
	I53	1.01±0.25
Two-species F_1's:		
D. sandwicense X *D. uncinatum*		
S11 X U22		0.95±0.07
S11 X U62		4.08±0.39
S21 X U82		2.10±0.41
S31 X U22		0.83±0.17
S51 X U42		4.61±0.31
D. sandwicense X *D. intortum*		
S11 X I23		26.83±1.95
S31 X I23		36.85±3.10
S31 X I43		2.98±0.41
Three-species F_1's:		
(S11xI23)(S11xU62)	1214	40.85±3.76
(S11xI23)(S31xU22)	1312	7.88±1.15
	1712	37.23±1.46
	1812	12.47±1.32
	2012	36.70±2.18
	2213	62.17±4.59

Table 25. Percentage of Pollen Abortion among the Three *Desmodium* Species and Their Hybrids (Continued)

Plant		Pollen Abortion (%) Mean±S.E.
(S11xI23)(s41xU62)	1414	36.13±0.91
(S21xI23)(S11xU22)	1914	9.27±0.68
	1915	1.71±0.36
(S21xI23)(S21xU72)	2115	2.97±0.37
	2215	18.56±1.51
(S21xI53)(S51xU42)	1417	45.15±2.01
(S21xI53)(S31xU22)	1216	1.12±0.23
	1416	4.99±0.26
	1516	11.49±1.08
	1616	1.58±0.28
	1716	2.81±0.33
	1816	3.44±0.51
	1916	2.77±0.23
	2116	1.12±0.11
	2216	1.51±0.06
(S21xI53)(U52xI33)	1217	5.56±0.31
	1218	4.08±0.33
	1317	18.55±2.10
	1318	6.90±0.83
(S21xU82)(S21xI53)	2313	1.83±0.41
(S31xI23)(U52xI33)	2612	48.70±3.81
(S51xI53)(S11xU22)	1419	45.15±2.10
	1519	58.19±2.09

Table 25. Percentage of Pollen Abortion among the Three Desmodium Species and Their Hybrids (Continued)

Plant		Pollen Abortion (%) Mean±S.E.
(S51xI23)(S31xU22)	1617	7.04±0.55
	1717	12.71±1.36
	1718	5.33±1.01
	1818	5.58±0.55
	1819	5.73±0.18
	1919	47.80±1.87
	1920	46.79±1.66
(S51xI23)(S31xU22)	1219	23.13±2.02
(S51xI23)(U52xI33)	1918	13.17±0.84
	2018	3.85±0.15
	2019	6.50±0.52
	2020	7.43±0.51
	2117	8.04±0.42
	2118	21.92±0.91
	2119	0.48±0.01
	2217	14.15±2.15
	2218	3.85±0.13
	2219	1.62±0.31

Table 26. Average Internode Length of Stems of Parental Clones, and Their F_1 and F_1 X F_1 Hybrids

Measurements made in the field during February, 1967.

Plant		Internode Length (cm) Mean±S.E.	Coefficient of Variance (%)
Parental clones:			
D. sandwicense	S11	3.2±.21	21
	S21	3.0±.17	17
	S31	2.9±.19	20
D. uncinatum	U12	6.2±.45	23
	U22	7.0±.51	23
	U32	6.6±.40	19
	U42	7.5±.61	26
	U72	7.3±.39	17
	U82	8.1±.70	27
D. intortum	I23	3.4±.16	22
	I33	4.8±.32	21
	I43	6.3±.28	14
	I53	4.7±.21	14
	I63	5.5±.43	25
Two-species hybrids:			
(S11xU22)		4.1±.31	24
(S11xU62)		3.1±.26	26
(S21xU72)		3.8±.34	31
(S21xU82)		3.6±.30	26
(S31xU22)		4.1±.33	25
(S31xU42)		3.8±.36	30
(S51xU42)		4.3±.39	29
(S11xI23)		4.4±.33	24
(S21xI23)		3.8±.27	23
(S21xI33)		4.5±.24	17
(S31xI23)		6.4±.59	29
(S51xI23)		5.0±.30	19

Table 26. Average Internode Length of Stems of Parental Clones, and Their F_1 and F_1 X F_1 Hybrids (Continued)

Plant		Internode Length (cm) Mean±S.E.	Coefficient of Variance (%)
(U52xI33)		5.1 .30	30
(I13xI33)		4.7 .37	24
Three-species hybrids:			
(S11xI23)(S31xU22)	1212	6.8 .77	36
	1312	5.0 .45	28
	1412	7.1 .88	38
	1512	5.3 .54	32
	1612	6.7 .38	18
	1712	8.4 .71	27
	1812	5.9 .54	28
	1912	2.2 .17	24
	2012	8.5 .81	30
	2112	5.2 .25	15
	2212	5.5 .28	16
	1213	5.9 .52	28
	1413	5.9 .28	15
	1613	6.1 .47	24
	1713	4.2 .39	29
	1813	3.6 .21	18
	1913	6.4 .41	20
	2013	4.9 .47	30
	2113	2.2 .15	21
(S21xI23)(S11xU22)	1914	5.0 .39	25
	2014	4.6 .41	28
	2015	5.4 .51	30
(S21xI23)(S21xU72)	2114	2.9 .19	21
	2214	5.3 .31	18
	2115	5.3 .31	18

Table 26. Average Internode Length of Stems of Parental Clones, and Their F_1 and F_1 X F_1 Hybrids (Continued)

Plant		Internode Length (cm) Mean±S.E.	Coefficient of Variance (%)
(S21xI53)(S31xU22)	1216	8.2±.40	15
	1316	7.4±.51	22
	1416	5.3±.43	26
	1516	6.0±.49	26
	1616	3.3±.17	16
	1716	3.3±.17	16
(S51xI23)(S31xU22)	1617	5.7±.51	28
	1717	5.6±.29	16
	1817	4.8±.41	27
	1618	5.7±.59	33
	1818	5.6±.44	25
	1810	3.1±.30	30
	1919	6.4±.60	30
	1920	1.8±.25	43
(S11xI23)(S41xU62)	1414	4.7±.21	14
	1415	1.4±.14	31
(S21xI23)(S31xU22)	1814	4.3±.31	23
	1715	0.7	
(S21xI53)(S51xU42)	1417	5.8±.42	23
(S51xI23)(S11xU62)	1619	0.6	
(S51xI23)(S11xU22)	1410	3.2±.33	32
	1519	3.2±.33	32
(S21xI53)(U52xI33)	1217	5.5±.51	30
	1317	6.0±.21	11
	1218	4.0±.23	15
	1318	4.0±.23	15

Table 26. Average Internode Length of Stems of Parental Clones, and Their F_1 and F_1 X F_1 Hybrids (Continued)

Plant		Internode Length (cm) Mean±S.E.	Coefficient of Variance (%)
(S31xI23)(U52xI33)	2613	4.9±.38	24
(S51xI23)(U52xI33)	2117	1.5±.13	27
	2217	4.0±.27	17
	1918	5.1±.48	30
	2018	4.0±.32	21
	2118	4.6±.21	15
	2218	4.0±.30	25
	2019	3.4±.17	16
	2219	4.7±.42	28
	2020	3.7±.31	26
(S21xU22)(S21xI53)	2312	3.3±.30	28
	2412	3.2±.25	25
	2512	3.1±.31	31
	2413	3.7±.19	16
	2513	2.9±.14	15

Table 27. Leaflet-size Indices (Leaflet Length x Width) and Ratios of Leaflet Length to Width of the Parental Clones and Their Hybrids

Plants		Leaflet-size indices	Ratios of length to width
Parental Clones:			
D. sandwicense	S11	10.15	1.84
	S21	8.04	1.76
	S31	12.58	1.89
D. uncinatum	U12	26.96	1.86
	U22	21.33	1.80
	U32	24.91	1.84
	U42	22.96	1.81
	U62	22.63	1.79
	U82	21.66	1.77
D. intortum	I13	21.88	1.62
	I23	18.44	1.59
	I33	32.01	1.49
	I43	21.78	1.58
	I53	26.45	1.56
	I63	21.77	1.55
Hybrid within *D. intortum*:			
I13xI33		31.32	1.51
Two-species hybrids:			
D. sandwicense x *D. uncinatum*			
S11xU62		8.53	1.79
S21xU72		10.38	1.72
S21xU82		7.00	1.71
S31xU22		11.41	1.77
S51xU42		11.50	1.77
D. sandwicense x *D. intortum*			
S11xI23		28.51	1.66
S21xI23		24.71	1.63
S21xI53		28.63	1.65
S31xI23		30.72	1.55
S31xI43		24.61	1.52

Table 27. Leaflet-size Indices (Leaflet Length x Width) and Ratios of Leaflet Length to Width of the Parental Clones and Their Hybrids (Continued)

Plants		Leaflet-size indices	Ratios of length to width
Three-species hybrids:			
(S11xI23)(S31xU22)	1212	14.25	1.58
	1312	9.41	1.58
	1412	14.91	1.61
	1512	26.16	1.52
	1612	14.70	1.47
	1712	12.87	1.47
	1812	22.93	1.47
	1912	9.07	1.42
	2012	13.79	1.62
	2112	22.72	1.91
	2212	16.91	1.71
	1213	19.40	1.82
	1413	15.38	1.34
	1513	13.67	1.48
	1613	18.01	1.70
	1713	15.12	1.58
	1813	20.38	1.67
	1913	10.68	1.89
	2013	22.91	1.70
	2113	8.07	1.79
(S21xI53)(S31xU22)	1216	20.03	2.02
	1316	9.62	2.06
	1416	9.47	2.17
	1516	18.27	1.78
	1616	16.01	1.85
	1716	12.35	2.06
	1816	16.21	2.01
	1916	13.76	2.20
	2016	11.96	2.14
	2116	11.42	2.27
	2216	12.20	2.51

Table 27. Leaflet-size Indices (Leaflet Length x Width) and Ratios of Leaflet Length to Width of the Parental Clones and Their Hybrids (Continued)

Plants		Leaflet-size indices	Ratios of length to width
(S21xI23)(S11xU22)	1914	9.40	1.54
	2014	11.87	1.41
	1915	11.63	1.83
	2015	17.17	1.50
(S21xI23)(S21xU72)	2114	15.40	1.88
	2214	16.20	1.58
	2115	18.32	1.76
	2215	11.69	1.99
(S21xU82)(S21xI53)	2312	8.08	1.86
	2412	9.38	1.82
	2512	7.70	1.67
	2313	6.79	1.76
	2413	6.51	1.80
	1513	6.73	1.59
(S11xI23)(S11xU62)	1214	10.95	1.64
(S11xI23)(S41xU62)	1414	13.25	2.03
	1415	7.46	1.64
(S21xI53)(S51xU42)	1417	17.65	1.64
(S51xI23)(S11xU22)	1419	14.40	1.94
	1519	16.16	2.07
	1520	11.34	2.31
(S31xI23)(U52xI33)	2612	3.15	2.06
(S21xI53)(U52xI33)	1217	12.59	1.59
	1218	10.81	1.68
	1318	12.32	1.67

Table 27. Leaflet-size Indices (Leaflet Length x Width) and Ratios of Leaflet Length to Width of the Parental Clones and Their Hybrids (Continued)

		Leaflet-size indices	Ratios of length to width
(S51xI23)(U52xI33)	2117	5.40	1.83
	2217	24.90	1.66
	1918	22.79	1.48
	2018	20.00	2.04
	2118	17.21	1.65
	2218	19.75	2.15
	2019	9.04	1.65
	2119	21.95	1.63
	2219	18.70	1.55
	2020	15.82	1.61
(S51xI23)(S31xU22)	1219	13.98	1.54
	1220	10.95	1.34
	1617	13.95	1.90
	1717	15.16	1.64
	1817	24.00	1.59
	1618	29.21	1.82
	1818	13.65	2.01
	1819	18.68	1.76
	1919	13.61	1.75
	1820	17.52	1.67
	1920	10.21	1.72
(S51xI33)(U52xI23)	2912	13.87	1.65

Table 28. Raceme Lengths of the Parental Clones, Their F_1 and F_1 X F_1 Hybrids

Plant		Raceme Length (cm) Mean±S.E.	Range
Parental clones:			
D. sandwicens	S11	13.7±0.9	10.0-16.0
	S21	15.0±1.0	11.0-18.0
	S31	14.9±1.2	12.0-22.0
D. uncinatum	U22	24.5±2.5	17.5-30.5
	U32	26.0±2.4	19.0-39.0
	U62	22.6±1.5	20.0-28.0
	U82	24.5±1.7	18.5-29.5
D. intortum	I23	10.8±1.0	8.5-14.0
	I43	14.6±0.8	12.0-16.0
	I53	11.0±1.1	8.0-15.0
Two-species hybrids:			
S11xI23		15.2±0.4	14.0-17.0
S21xI23		13.3±0.8	9.0-14.5
S31xI23		19.8±0.7	18.0-22.0
S51xI23		13.7±0.9	9.0-15.0
S21xI53		14.9±1.0	10.5-17.5
S11xU22		14.0±1.5	11.0-17.0
S11xU62		13.6±1.8	10.0-17.0
S21xU82		15.5±0.5	14.0-17.0
S31xU22		17.0±2.0	15.0-24.0
S51xU42		14.2±0.4	13.5-15.0
Three-species hybrids:			
(S11xI23)(S31xU22)	2213	8.7±0.8	7.0-11.0
(S21xI23)(S11xU22)	1914	17.0±0.8	15.0-19.0
(S21xU52)(S31xU22)	1216	14.4±1.2	11.0-17.5
(S21xU82)(S21xU52)	2313	12.8±1.6	10.0-18.0
(S21xI23)(S21xU72)	2215	9.6±1.8	5.0-15.0
(S21xI53)(S31xU22)	2216	13.5±1.2	10.5-17.0
(S21xI53)(U52xI33)		16.5±2.3	9.5-24.0
(S31xI23)(U52xI33)		15.6±1.6	13.5-22.0
(S51xI23)(S31xU22)		20.2±1.5	17.0-23.5
(S51xI23)(U52xI33)		45.0±0.9	43.0-48.0
(S51xI23)(S11xU22)		14.2±1.5	9.0-17.0

Table 29. Green Weights and Dry Matter Percentages of the Parental Clones, Their F_1 and F_1 X F1 Hybrids

Plant		Green weights in grams			Dry matter percentages		
		1st harvest	2nd Harest	Average	1st harvest	2nd harvest	average
Parental clones:							
D. sandwicense	S11	50.6	56.0	53.3	29.1	31.7	30.4
	S21	108.6	159.2	133.9	27.8	29.1	28.5
	S31	31.2	34.6	32.9	28.0	29.9	29.0
D. uncinatum	U12	446.0	53.6	249.8	25.2	36.8	31.0
	U42	268.0	49.4	158.7	25.6	37.0	31.3
	U62	285.8	54.8	170.3	26.5	34.4	30.5
D. intortum	I13	142.8	493.2	318.0	25.4	24.3	24.8
	I23	46.8	91.8	69.3	27.8	26.9	27.4
	I43	82.8	214.4	148.6	25.3	23.2	24.3
	I53	469.4	664.8	567.1	22.9	23.2	23.1
	I63	41.0	102.4	71.7	25.4	24.5	25.0
F_1 hybrids:							
S31xU42		67.2	114.6	90.9	27.7	25.3	28.4
S11xI23		319.2	505.6	412.4	24.5	24.1	24.9
S21xI53		382.6	488.6	435.6	23.0	24.1	23.6
S31xI53		143.2	315.4	229.3	24.3	26.2	25.3
I13xI33		517.8	707.0	612.4	22.9	23.4	23.2

Table 29. Green Weights and Dry Matter Percentages of the Parental Clones, Their F_1 and F_1 X F_1 Hybrids (Continued)

Plant		Green weights in grams			Dry matter percentages		
		1st harvest	2nd harvest	average	1st harvest	2nd harvest	average
F_1 X F_1 hybrids:							
(S11xI23)(S31xU22)	1213	292.0	118.6	205.3	23.9	29.7	26.8
(S21xI53)(S31xU22)	1216	185.2	264.6	224.9	23.3	25.3	24.3
(S21xI53)(S31xU22)	1316	220.4	181.2	200.8	24.3	27.7	26.0
(S11xI23)(S31xU22)	1512	282.4	461.8	572.1	24.4	24.7	24.6
(S51xI23)(S31xU22)	1618	415.8	407.0	411.4	22.8	24.7	23.8
(S51xI23)(S31xU22)	1717	421.2	180.8	301.0	23.5	25.4	24.5
(S11xI23)(S31xU22)	1812	306.4	258.8	296.1	24.7	27.4	26.1
(S11xI23)(S31xU22)	1813	187.8	142.0	164.9	24.1	24.3	24.2
(S51xI23)(S31xU22)	1818	82.0	191.0	136.5	25.3	27.4	26.4
(S51xI23)(S31xU22)	1819	382.0	406.6	394.3	24.2	27.1	25.6
(S51xI23)(S31xU22)	1919	141.8	310.4	226.1	25.2	25.5	25.4
(S21xI23)(S11xU22)	2014	176.8	223.6	200.2	25.0	26.7	25.8
(S21xI23)(S11xU22)	2015	182/4	227.0	204.7	23.2	25.2	24.2
(S21xI53)(S31xU22)	2016	277.6	234.4	256.0	24.9	27.6	26.3
(S21xI23)(S21xU72)	2214	324.0	350.0	337.0	23.1	24.1	23.6
(S21xI23)(U52xI53)		571.2	522.0	546.6	21.4	24.9	23.2

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